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Groups of male and female Fischer 344 rats were dosed with tetralin and indan over a 14 day period. Histopathology of the rat kidneys revealed significant damage to the proximal tubules of the male rats dosed with the hydrocarbons. Control male rats dosed with water and female rats dosed with hydrocarbons or water showed no renal damage. The urinary metabolites identified for tetralin included 1-tetralol, 2-tetralol, 2-hydroxy-1-tetralone, 4-hydroxy-1-tetralone, 1,2-tetralindiol, and 1,4-tetralindiol. The indan urinary metabolites found were 1-indanol, 2-indanol, 5-indanol, 1-indanone, 2-indanone, 1-hydroxy-3-indanone, 1-hydroxy-2-indanone and 1,2-indandiol.

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THE STUDY OF NEPHROTOXICITY AND METABOLISM OF TETRALIN AND INDAN IN FISCHER 344 RATS

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The overview and conclusions contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies or endorsements, either expressed or implied, of the Air Force Office of Scientific Research or the U.S. Government.

Summary

Certain hydrocarbons, both acyclic and cyclic, have been shown to induce a nephrotoxic effect in male rats. There is a strong feeling that the renal damage may be related to the metabolic handling of the hydrocarbon by the animals. Both cis- and trans-decalin as well as other saturated cyclic molecules have shown a proclivity of inducing the nephrotoxicity. Tetralin and indan, because of the aromatic ring which exists as part of their structures, introduces a structural difference when compared to other cyclic hydrocarbons which have produced the hyaline droplet nephrotoxicity.

Male and female Fischer 344 rats were dosed intragastrically with tetralin or indan on an every other day regimen for 14 days. When compared with male control rats dosed with water, the male rat exposed to tetralin and indan exhibited increased cytoplasmic hyaline droplets in proximal convoluted tubular epithelial cells, which were indicative of toxic injury. Additionally, foci of cellular degeneration were present within proximal convoluted tubules. Exposed and control female Fischer 344 rats did not display any renal damage. The nephrotoxic effects of tetralin were equivalent to the damage done by the decalins. Indan, however, produced less renal toxicity. The planar structure of indan apparently does not interfere to the same extent with the degradation of alpha 2u-globulin and thus produces proportionately less kidney damage.

The tetralin metabolites found in the urine of all male and female exposed rats were: 1-tetralol, 2-tetralol, 2-hydroxy-1-tetralone, 4-hydroxy-1-tetralone, 1,2-tetralindiol and 1,4-tetralindiol. Tetralin metabolites were not found in the kidney extracts of male and female rats dosed with tetralin.

The urinary metabolites of indan found in all male and female dose rats were: 1-indanol, 2-indanol, 1-indanone, 2-hydroxy-1-indanone, 3-hydroxy-1-indanone, cis- and trans- 1,2-indandiol. 1-Indanol and 1-indanone were found in the kidney extracts of all male dosed 344 rats.

I. INTRODUCTION

In the early 1980s, widespread use of petroleum products and public emphasis on toxic hazard assessment influenced the American Petroleum Institute to sponsor a study to determine what potential adverse health effects, if any, were presented to people who came in contact with gasoline under normal circumstances. The study, conducted by the International Research and Developemnt Corporation, examined the inhalation effects of unleaded gasoline in mice and rats. The unexpected occurrence of dose-related degrees of kidney damage, including renal neoplasia, in male rats evoked concern. Renal lesions were not observed in female rats or in males or females of other test species.

The results of the gasoline study, reported in 1983 by the Universities Associated for Research and Education in Pathology, prompted extensive investigations by the scientific community on unleaded gasoline and other hydrocarbon-based compounds in an attempt to ascertain the mechanism of nephrotoxicity in the male rat. In 1983, a Workshop on the Kidney Effects of Hydrocarbons was conducted in Boston. The material presented during this workshop is published in a book entitled "Renal Effects of Petroleum Hydrocarbons" (Advances in Modern Environmental Toxicology, Vol VII, M.A. Mehlman, ed., Princeton Scientific Publishers, Inc., 1984). The concluding remarks from this workshop urge the continuation of investigations of nephrotoxic hydrocarbons. Additionally, at the 1985 meeting of the Society of Toxicology, a continuing education course in renal toxicology, with special emphasis on hydrocarbon nephrotoxicity was offered.

In 1985, The American Cancer Society estimated that 19,700 Americans, approximately two-thirds of them male, would develop renal cancer and 8,900 would die from the disease. The relevance of nephropathy observed in male rats exposed to various hydrocarbons to the occurrence of renal neoplasia in man is of concern.

Anatomic and physiologic differences, especially between the rat and man, were observed when normal kidneys from animals of different species were compared. However, these comparative studies have not yet explained why, of all the animal species studied, only male rats develop kidney lesions and renal carcinomas. Therefore, the suitability of using the male rat as a model for human risk asessment is in question.

The observation of hydrocarbon-induced nephrotoxicity and carcinogenicity in male rats raises important questions regarding the potential hazard of numerous hydrocarbon-based compounds. Despite the widespread use of petroleum products, the potential health effects of many hydrocarbons remain largely uncharacterized. Therefore, to aid in the elucidation of a mechanism of toxicity, it is imperative that experiments be conducted to evaluate the structure-activity relationships of various hydrocarbons and their involvement in nephrotoxicity.

NORMAL RENAL STRUCTURE AND FUNCTION

A discussion of normal renal structure and function is necessary in order to understand hydrocarbon-induced nephrotoxicity. The kidney and its components are diagrammed in Figure 1. If a kidney is sectioned sagittally, two regions can be distinguished, an outer cortex and an inner medulla. A transitional area called the corticomedullary junction exists between the cortex and the medulla where elements from both regions are found. The medulla is shaped like an inverted pyramid which has its base juxtaposed to the inner-most border of the cortex and its apex (papilla) facing the pelvis. Each medullary pyramid and the cortical tissue that encase it constitute a lobe. Some species, including the rat, have unilobular kidneys where the narrow papilla projects into the flared end of the ureter, known as the renal pelvis.

The kidney is a compound gland formed of uriniferous tubules. Each uriniferous tubule is composed of a nephron and a collecting duct system. The nephron produces the urine while the collecting duct system collects, concentrates, and transports this fluid to the pelvis, where it leaves the kidney. The nephron consists of a Bowman's capsule, a proximal convoluted tubule, an ascending and descending loop of Henle, and a distal convoluted tubule.

Histologic evaluation of the renal cortex and the renal medulla displays regular structural patterns. Sections of the renal cortex show bands called medullary rays which consist of descending and ascending limbs of the loop of Henle and straight collecting tubules.

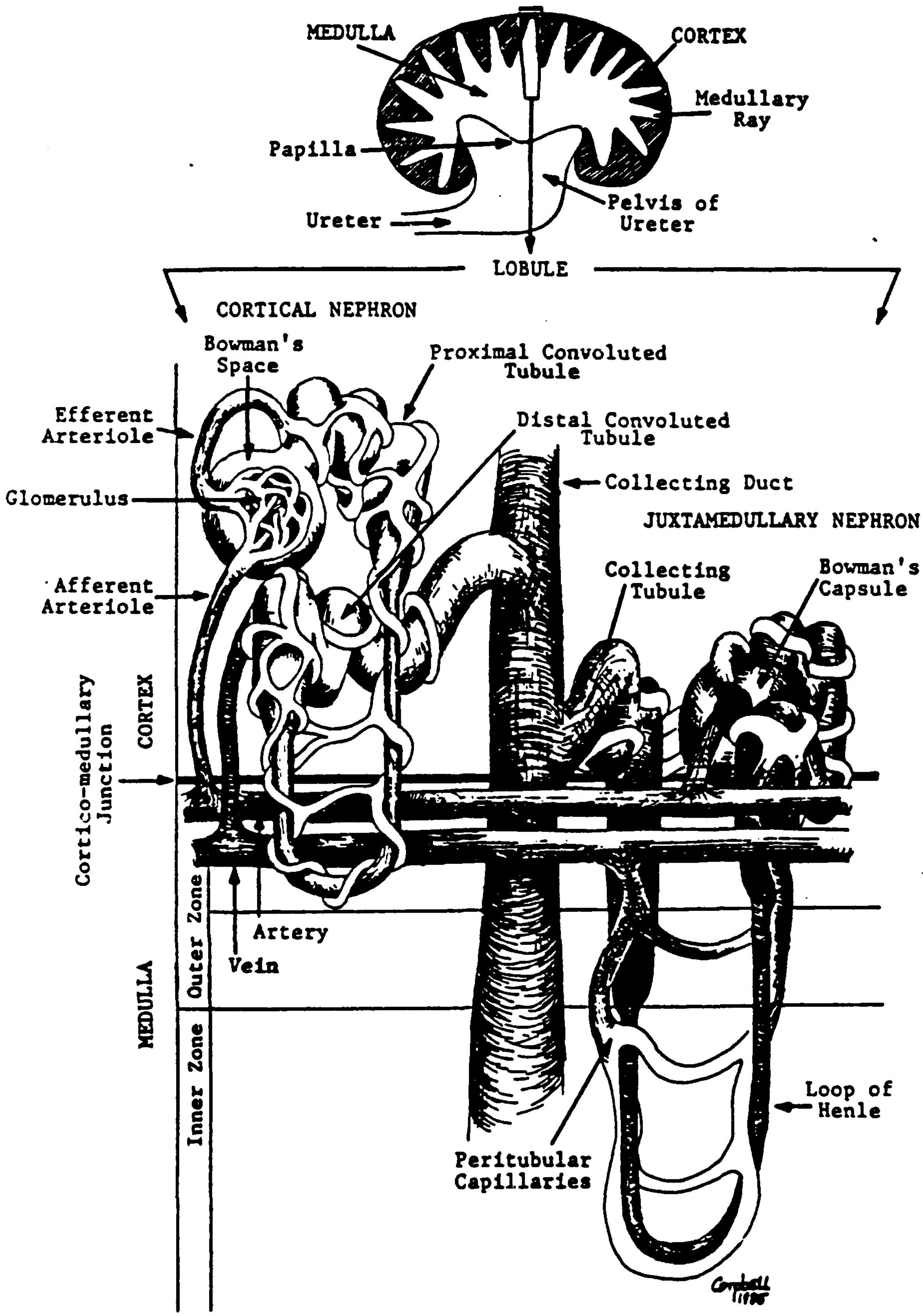


FIGURE 1. SCHEMATIC DIAGRAMS OF A UNILOBULAR KIDNEY.

Medullary sections reveal a visible separation of the medulla into outer and inner zones. The outer zone contains the loops of Henle of short nephrons and straight collecting tubules while the inner zone contains the loops of Henle of long nephrons and straight collecting ducts and papillary ducts.

The nephron is the functional unit of the kidney and contains within Bowman's capsule a special filtering mechanism called the glomerulus. The glomerulus is supplied with an arteriole of the renal artery. The arteriole enters Bowman's capsule as the afferent arteriole and branches into a network of capillaries which form part of the glomerulus. These capillaries rejoin and emerge from Bowman's capsule as the efferent arteriole. The efferent arteriole divides into a system of capillaries (peritubular capillaries) which tightly surrounds all of the tubular parts of the nephron. Blood in the peritubular capillaries drains into venules, which combine to form the renal vein.

As blood flows through the capillaries of the glomerulus, the pressure of the blood causes fluid to filter into Bowman's capsule and then into the proximal tubule. The function of the different segments of the kidney tubule is to reabsorb certain substances, in varying degrees, depending on the needs of the body. In the proximal tubule there is active transport of filtered glucose, amino acids, sodium, and ions from the tubule into the proximal tubular epithelial cells. These solutes are processed inside the epithelial cells, and transported into the peritubular capillaries. The result is that water passes out of the tubule by osmosis while urea and other wastes are concentrated in the tubule. Also, low molecular weight proteins that were filtered at the glomerulus are transported into the proximal tubular epithelium where lysosomes degrade the proteins into their constituent amino acids. These amino acids diffuse through the basal membrane of the cell, and into the peritubular capillaries.

In the loop of Henle a counter-current fluid mechanism and the presence of various hormones increase the concentration of sodium chloride in the tubular fluid. As the tubular fluid flows into the distal tubule the permeability of the distal tubule and collecting duct is increased under hormonal control and reabsorption of water follows by osmosis. The result is that fluid leaving the collecting duct to enter the pelvis of the kidney is concentrated urine.

LESIONS CHARACTERIZING HYDROCARBON-INDUCED NEPHROTOXICITY

Male rats exposed to some hydrocarbons develop dose-related nephropathies which are not observed in female rats and control rats or in the males and females of other animal species. These lesions seem to be dependent on the progressive accumulation of excessive quantities of resorbed, undigested protein in the cytoplasm of proximal tubular cells (Bruner et al., 1983). This undigested protein usually appears as a spherical, homogeneous body termed a hyaline droplet. The inability of the cell to efficiently degrade and export these resorbed protein droplets results in greatly engorged phagolysosomes which precede cell death and exfoliation (Bruner et al., 1983). In contrast, most other nephrotoxins exert their effects by interfering with essential metabolic processes, causing cell damage without excessive accumulations of resorbed proteins (Cheville, 1983). An overview of the pathologic findings from the kidneys of laboratory animals subjected to acute, subchronic, and chronic hydrocarbon exposures is presented.

A. Acute Exposures: Male rats exposed to certain hydrocarbons for up to 14 days develop excessive cytoplasmic hyaline droplets in the cells of the proximal convoluted tubules. When exposures are continued for more than 14 days, renal tubules near the corticomedullary junction accumulate cellular debris and cortical segments exhibit hyperplastic changes.

B. Subchronic Exposures: Most subchronic exposures have been based on the 90-day continuous inhalation of a specified hydrocarbon fuel. Male rats sacrificed immediately following various subchronic exposures exhibit a distinct increase in cytoplasmic hyaline droplets in the proximal tubular epithelial cells throughout the cortex. Additionally, tubular segments near the corticomedullary junction are focally dilated and filled with coarsely granular, eosinophilic debris. These dilated, plugged tubular segments are thought to represent that region of the proximal tubule where it narrows to enter the descending limb of the loop of Henle (Bruner et al., 1983). Transmission electron microscopy has demonstrated that tubular plugs consist of cell debris and that hyaline droplets are compatible with membrane-bound accumulations of protein in phagolysosomes. Other renal structures, including glomeruli, are morphologically unremarkable with both light and electron microscopy. Pathologic evaluation of animals subchronically exposed and then held for long-term, post-exposure evaluation revealed tubular degeneration consistent with "old-rat nephropathy" (explained below).

C. Chronic Exposures: Many chronic hydrocarbon studies have consisted of one-year intermittent inhalation exposures. Histopathologic examination of the male rat kidneys following these one-year exposures has revealed a significant increase in primary renal tumors. Other kidney changes noted were an increase in lesions typical of "old-rat nephropathy".

One problem inherent in long-term nephrotoxicity studies is that lesions known as "old-rat nephropathy" often obscure pathologic evaluations. "Old-rat nephropathy" is a common degenerative kidney disease predominantly seen in the male rat. By careful examination of tissues, differences between "old-rat nephropathy" and hydrocarbon-induced nephropathy can be distinguished. Foremost among these differences is that degenerative changes are more severe in hydrocarbon exposed male rats. This increased severity is accompanied by the presence of mineralized debris in the medullary tubules and hyperplasia of the surface epithelium over the renal papillus which usually is not present in "spontaneous" "old-rat nephropathy" (Bruner et al., 1983).

PREVIOUS HYDROCARBON NEPHROTOXIC STUDIES

Carpenter et al. (1975, 1977) studied animal responses to several solvents such as Stoddard solvent, a petroleum distillate composed of straight and branched chain hydrocarbons, naphthenes, and benzene derivatives. In each study, single to multiple low doses of solvent produced noticeable renal lesions in the kidneys of male rats compatible with those observed in male rats acutely exposed to hydrocarbons.

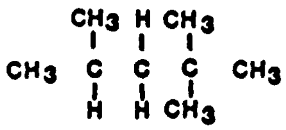
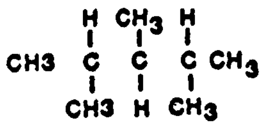
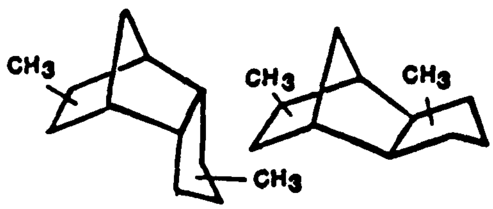
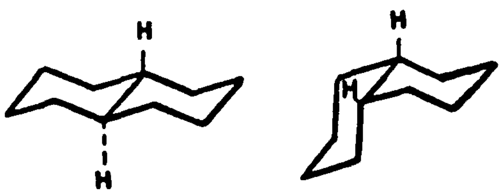
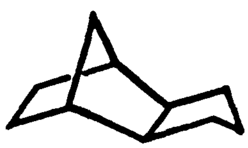
General toxicology studies conducted at the Harry G. Armstrong Aerospace Medical Research Laboratory (AAMRL) evaluated petroleum and shale-derived JP-5, a jet fuel composed of aliphatic and aromatic hydrocarbons with the majority of the straight-chain hydrocarbons being between C_{10} and C_{15} (Gaworski, 1979). Purebred beagles, rats, and mice were continuously exposed in inhalation chambers to 150 or 750 mg/m^3 of JP-5 for 90 days. Both males and females of each species were exposed, along with equal numbers of controls. Following exposure, all of the dogs and one-third of the rodents from each group were sacrificed. The remaining rodents were held under observation for 19 months. At that time, one-half of the rodents were sacrificed and the other half were held until the mortality of each group reached 90%. Histopathologic evaluations were performed on all animals and renal changes, noticed only in the male rats, were similar to the lesions of male rats subchronically exposed to other hydrocarbon-based compounds.

Since this initial short-term study, the U.S. Air Force with the U.S. Navy and the Toxic Hazards Research Unit at AAMRL, have conducted numerous investigations of distillate propellants (McNaughton et al., 1983). JP-8, JP-4, and diesel fuel marine (DFM) are several petroleum and shale-derived distillates which have been studied in 90-day inhalation experiments. JP-4 is a low boiling point, highly volatile hydrocarbon mixture much like gasoline. JP-8 is a mixture of hydrocarbon of intermediate boiling point and volatility and is similar to the civilian jet fuel, A-1. DFM is a mixture of long chain aliphatic hydrocarbon compounds with a small portion of aromatic hydrocarbons. For those studies in which pathologic evaluations have been completed, the kidney lesions observed were similar to those lesions found in animals subchronically exposed to hydrocarbon-based compounds.

Studies conducted to characterize the nephrotoxic activity of unleaded gasoline in the male rat indicated that the toxic insult occurred predominantly from the fractions containing saturated, branched aliphatic compounds (Halder et al., 1984). Therefore, numerous studies have been initiated to determine the toxicity of "pure" hydrocarbons. The majority of the exposures performed have been either short-term oral dosings or long-term inhalation exposures.

Short-term oral dosings have been performed using 2,2,4-trimethylpentane, and 2,3,4-trimethylpentane as well as the cyclic hydrocarbons RJ-4 fuel (perhydromethylcyclopentadiene), JP-10 fuel (tricyclodecane), and decalin (decahydronaphthalene). Kidneys from male rats exposed by gavage to these pure hydrocarbons have exhibited the same pathologic changes as seen with the distillate fuels (Bruner, et al., 1983). Table 1 summarizes the results from short-term exposure of male Fischer 344 rats to various hydrocarbons.

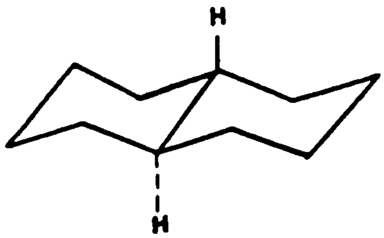
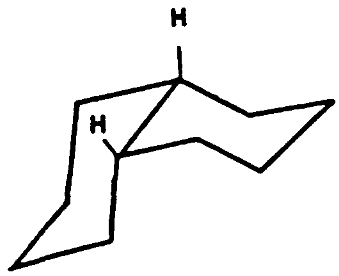
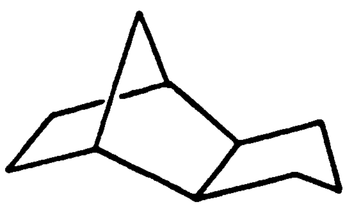
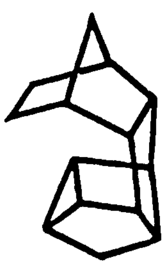
TABLE 1. SHORT-TERM ORAL EXPOSURE OF MALE FISCHER 344 RATS TO SPECIFIC (PURE) HYDROCARBONS

AGENT	DOSE	EXPOSURE LENGTH	RENAL LESIONS
2,2,4-Trimethylpentane 	0.09,0.3, 1.0 mL/kg	8 doses over 24 Days	Hyaline droplets and necrosis of proximal tubular epithelium. Impacted cellular detritus at the corticomedullary junction.
2,3,4-Trimethylpentane 	0.09,0.3, 1.0 mL/kg	8 doses over 24 Days	Hyaline droplets and necrosis of proximal tubular epithelium. Impacted cellular detritus at the corticomedullary junction.
RJ-4 Fuel 	0.09,0.3, 1.0 mL/kg	8 doses over 24 Days	Hyaline droplets and necrosis of proximal tubular epithelium. Impacted cellular detritus at the corticomedullary junction.
Decalin 	2.5 g/kg	7 doses over 14 Days	Hyaline droplets and necrosis of proximal tubular. epithelium. Impacted cellular detritus at the corticomedullary junction.
JP-10 	0.09,0.3, 1.0mL/kg	8 doses over 14 Days	Hyaline droplets in proximal tubular epithelium.

Adapted From: Bruner, R. H., and Pitts, L. L.: Nephrotoxicity of hydrocarbon propellants to male Fischer-344 rats. Proc 13th Ann Conf Environ Toxicol, Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, 337-349 (1983).

Long-term inhalation exposures have been accomplished primarily with the pure hydrocarbons, decalin, JP-10, and RJ-5 [endo-endo-dihydrodinorbornadiene] (Bruner et al., 1983). Table 2 summarizes the pathology observed after long-term inhalation exposure to pure hydrocarbons. Studies utilizing JP-10 and RJ-5 included one year intermittent inhalation exposures (6 hr/day for 5 days/wk) followed by long-term, post-exposure holding. As in the other hydrocarbon fuel studies, histopathologic evaluation of kidneys from animals exposed to JP-10 and RJ-5 displayed typical chronic hydrocarbon-induced nephrotoxicity in male rats. The most significant histopathologic finding in male rats exposed to JP-10 and held post-exposure was the presence of nine primary renal cell carcinomas and one poorly differentiated malignant renal neoplasm in 50 exposed rats as compared to only one renal cell carcinoma in controls. In animals exposed to RJ-5, a high incidence of renal tumors in male rats was also seen. Of the 65 male rats exposed to 150 mg/m^3, four renal cell adenomas and five renal cell carcinomas were found. Only one renal cell carcinoma was found 65 male rats exposed to 30 mg/m^3 of RJ-5. No renal cell tumors were identified in control animals. These data indicate that both JP-10 and RJ-5 cause renal cell tumor formation and nephrotoxicity in male rats. A 90-day inhalation experiment was conducted with decalin, and the hydrocarbon-induced nephrotoxicity observed was virtually identical to that of the 90-day distillate inhalation experiments.

TABLE 2. LONG-TERM INHALATION EXPOSURE OF MALE FISCHER 344 RATS TO SPECIFIC (PURE) HYDROCARBONS

AGENT	DOSE	EXPOSURE LENGTH	RENAL LESIONS
Decalin ($C_{10}H_{18}$)  	5,50 ppm	90 days continuous	Subchronic: Cytoplasmic hyaline droplets and necrosis of proximal tubular epithelium. Impacted tubules at corticomedullary junction. Oncogenic: Accentuated tubular degeneration, medullary mineralization and urothelial papillary hyperplasia.
JP-10 ($C_{10}H_{16}$) 	100 ppm	1 year industrial (6h/d, 5d/wk)	Ten primary renal cell carcinomas in 50 rats.
RJ-5 ($C_{14}H_{20}$) 	30,150 mg/m3	1 year industrial	Low Dose: One renal cell carcinoma. High Dose: Four renal cell adenomas and five renal cell carcinomas in 65 rats.

Adapted From: Bruner, R. H., and Pitts, L. L.: Nephrotoxicity of hydrocarbon propellants to male Fischer-344 rats. Proc 13th Ann Conf Environ Toxicol, Air Force Aerospace Medical Research Laboratory, Wright-Patterson AFB, Ohio, 337-349 (1983).

Recently, Loury (1987), using rat kidney cell cultures exposed to unleaded gasoline, found that unleaded gasoline did not evoke unscheduled DNA synthesis. This indicated that induced cell turnover may be an important factor in the carcinogenic action of this motor fuel.

Alden et al. (1983) used both short-term oral gavage treatments and intermittent inhalation exposures to characterize the effect of decalin on male rats. Using two-dimensional electrophoresis and immunofluorescent techniques, Alden et al. demonstrated hyaline droplet accumulation to consist of alpha 2u globulin a sex-dependent protein synthesized in the liver under testosterone induction and is found in the urine of young adult male rats (Irwin et al., 1971). Levels of alpha 2u globulin are extremely low in the female rat and undetected in humans at this time (Kloss, 1985). Alpha 2u globulin is a low molecular weight protein (18,000 to 20,000 daltons) and, after its synthesis in the liver, is filtered by the kidney glomeruli. A large portion of the filtered protein is then reabsorbed by the proximal convoluted tubules and catabolized by lysosomes into constituent amino acids. The increased appearance of alpha 2u globulin in the urine of male rats is thought to result from the inability of the proximal tubules to reabsorb the protein (Kloss, 1985). Alden et al. (1983) postulated that accumulation of hyaline droplets in epithelial cells is due to an alteration in the handling of alpha 2u globulin.

HYDROCARBON METABOLISM STUDIES

The structure of a xenobiotic determines the type of biotransformation it undergoes and also determines the intermediates or final products formed. The metabolite formed may react with cellular components instead of being excreted. Therefore, an understanding of the metabolic handling of various hydrocarbons is necessary in order to describe more fully the nature of hydrocarbon-induced nephrotoxicity. It is proposed that the interaction of hydrocarbon metabolites with indigenous proteins such as alpha 2u globulin might compromise tubular cell protein catabolism, resulting in the kidney lesions observed (Kloss et al., 1985). As a result, research has focused on the isolation and identification of hydrocarbon metabolites.

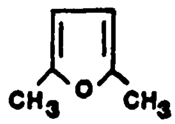
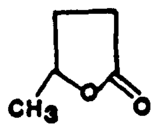
Exposures to n-hexane and n-heptane, major constituents of industrial solvent mixtures, have been shown to cause polyneuropathy in man (Casarett and Doull, 1980). Several general hydrocarbon toxicity studies have been performed with n-hexane and n-heptane to characterize their metabolism and role in neurotoxicity (Perbellini et al., 1982; Bahima et al., 1984). These studies have indicated that peripheral neuropathies can be attributed to the metabolite 2,5-hexanedione. However, no nephrotoxicity was reported in any of these straight-chain hydrocarbon toxicity studies. Other nephrotoxicity studies using acyclic aliphatic compounds have indicated that only saturated, branched compounds induce renal lesions in male rats (Kloss, 1985). The urinary metabolites of n-hexane and n-heptane are listed in Table 3. It is important to note that the primary metabolites of n-hexane and n-heptane are alcohols and hydroxyketones.

Olson et al. (1985) orally dosed Fischer 344 rats with the branched, aliphatic hydrocarbon, 2,2,4-trimethylpentane, every other day for 14 days. Trimethylpentane (TMP) is a major component of gasoline and the standard reference fuel for indicating "octane rating". Kidney tissues from TMP exposed animals manifested the same pathologic changes as observed in tissues from animals acutely exposed to distillate fuels. Analysis of urinary metabolites indicated that 2,2,4-trimethylpentane is excreted primarily as a carboxylic acid derivative and to a lesser degree as a monosubstituted alcohol. The urinary metabolites of 2,2,4-TMP are shown in Table 4. There were no hydroxyketones or diols detected in the urine of 2,2,4-TMP exposed rats.

A study by Charbonneau (1987) using $[C^{14}]$-2,2,4-TMP found that 2,4,4-trimethyl-2-pentanol was the major metabolite present in male rat kidneys, but absent in female rat kidneys. The renal retention of 2,4,4-trimethyl-2-pentanol appeared to account for the delayed clearance observed in the disposition of $[C^{14}]$-TMP derived radiolabel, reflecting the accumulation of a metabolite-α2u-globulin complex.

Although numerous studies have evaluated the metabolism of straight and branched chain hydrocarbons, only a few studies have been conducted to evaluate the metabolism of cyclic hydrocarbons. Inman et al. (1983) studied the metabolism of the C_{10} hydrocarbon fuel JP-10 in

TABLE 3. n-HEXANE AND n-HEPTANE METABOLITES IDENTIFIED IN THE URINE OF RATS

n-HEXANE METABOLITE	STRUCTURE
2-Hexanol	$CH_3CH_2CH_2CH_2\overset{\overset{OH}{\mid}}{C}H\ CH_3$
3-Hexanol	$CH_3CH_2CH_2\overset{\overset{OH}{\mid}}{C}H\ CH_2CH_3$
Methyl n-butyl ketone	$CH_3\overset{\overset{O}{\|}}{C}CH_2CH_2CH_2CH_3$
2,5-Hexanedione	$CH_3\overset{\overset{O}{\|}}{C}CH_2CH_2\overset{\overset{O}{\|}}{C}\ CH_3$
2,5-Dimethylfuran	
γ -Valerolactone	

n-HEPTANE METABOLITE	STRUCTURE
1-Heptanol	$CH_3CH_2CH_2CH_2CH_2CH_2\overset{\overset{OH}{\mid}}{C}H_2$
2-Heptanol	$CH_3CH_2CH_2CH_2CH_2\overset{\overset{OH}{\mid}}{C}HCH_3$
3-Heptanol	$CH_3CH_2CH_2CH_2\overset{\overset{OH}{\mid}}{C}H\ CH_2CH_3$
4-Heptanol	$CH_3CH_2CH_2\overset{\overset{OH}{\mid}}{C}H\ CH_2CH_2CH_3$

TABLE 3 (CONTINUED)

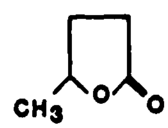
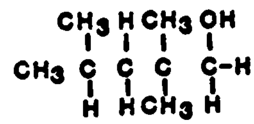
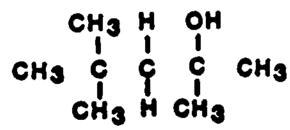
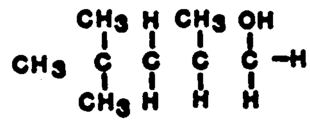
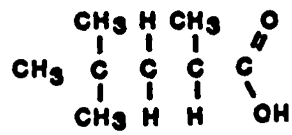
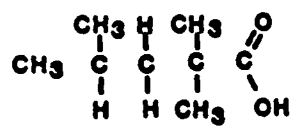
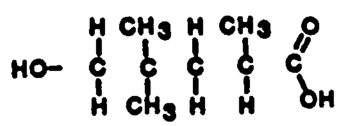
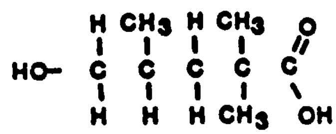
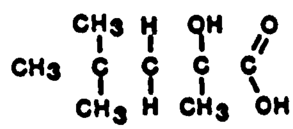
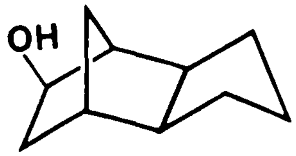
n-HEPTANE METABOLITE	STRUCTURE
2-Heptanone	$CH_3CH_2CH_2CH_2CH_2\overset{O}{\overset{\|}{C}}CH_3$
3-Heptanone	$CH_3CH_2CH_2CH_2\overset{O}{\overset{\|}{C}}CH_2CH_3$
2,5-Heptanediol	$CH_3CH_2\overset{OH}{\overset{\mid}{C}}H\ CH_2CH_2\overset{OH}{\overset{\mid}{C}}HCH_3$
2,6-Heptanediol	$CH_3\overset{OH}{\overset{\mid}{C}}H\ CH_2CH_2CH_2\overset{OH}{\overset{\mid}{C}}H\ CH_3$
5-Hydroxy-2-heptanone	$CH_3CH_2\overset{OH}{\overset{\mid}{C}}H\ CH_2CH_2\overset{O}{\overset{\|}{C}}CH_3$
6-Hydroxy-2-heptanone	$CH_3\overset{OH}{\overset{\mid}{C}}H\ CH_2CH_2CH_2\overset{O}{\overset{\|}{C}}CH_3$
6-Hydroxy-3-heptanone	$CH_3\overset{OH}{\overset{\mid}{C}}HCH_2\overset{O}{\overset{\|}{C}}CH_2CH_3$
2,5-Heptanedione	$CH_3CH_2\overset{O}{\overset{\|}{C}}CH_2CH_2\overset{O}{\overset{\|}{C}}CH_3$
2,6-Heptanedione	$CH_3\overset{O}{\overset{\|}{C}}CH_2CH_2CH_2\overset{O}{\overset{\|}{C}}CH_3$
γ-Valerolactone	

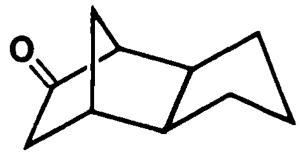
TABLE 4. 2,2,4-TRIMETHYLPENTANE METABOLITES IDENTIFIED IN THE URINE OF MALE RATS

2,2,4-TRIMETHYLPENTANE METABOLITE	STRUCTURE
2,2,4-Trimethyl-1-pentanol	
2,4,4-Trimethyl-2-pentanol	
2,4,4-Trimethyl-1-pentanol	
2,4,4-Trimethyl-1-pentanoic acid	
2,2,4-Trimethyl-1-pentanoic acid	
2,4,4-Trimethyl-5-hydroxy-1-pentanoic acid	
2,2,4-Trimethyl-5-hydroxy-1-pentanoic acid	
2,4,4-Trimethyl-2-hydroxy-1-pentanoic acid	

Fischer 344 male rats. The metabolites are shown in Figure 2. The only urinary metabolite isolated was 5-hydroxy-JP-10. Since JP-10 produced renal lesions in male rats, one kidney from each of the rats was removed and homogenized for metabolite analysis. The homogenized kidney extract yielded only one derivative of JP-10, 5-keto-JP-10. Renal damage was not detected in female Fischer 344 rats dosed with JP-10 and 5-keto-JP-10 was not isolated from their kidney extracts.



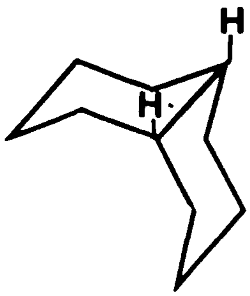
5-Hydroxy-JP-10



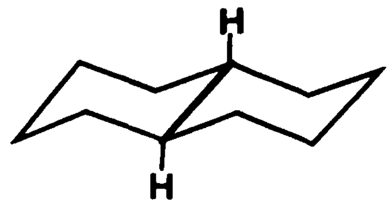
5-Keto-JP-10

FIGURE 2. JP-10 METABOLITES

Olson et al. (1985) studied the metabolism of cis- and trans-decalin in Fischer 344 rats. The stereoisomers of decalin are shown in Figure 3. Decalin is a component of motor fuels and lubricants and is a solvent for fats, resins, oils and waxes. Like JP-10, decalin is a cyclic hydrocarbon containing 10 carbons. Cis- and trans-decalin produced typical hydrocarbon nephrotoxicity in male rats, while female and control rats exhibited no renal pathology.



Cis-Decalin



Trans-Decalin

FIGURE 3. DECALIN STEREOISOMERS

Olson et al. identified and isolated the urinary metabolites of cis- and trans-decalin from male and female Fischer 344 rats. The relative amount of each metabolite detected is listed in Table 5. In both male and female Fischer 344 rats, the principal urinary metabolite of cis-decalin was cis,cis-2-decalol and the major urinary metabolite of trans-decalin was trans,cis-2-decalol. The principal metabolic difference between male and female rats treated with cis-decalin was the presence of cis,cis-1-decalol in the urine of male rats but not in the urine of female rats. It was also noted that the metabolite cis,trans-1-decalol, although found in the urine of both male and female rats, was present in relative larger quantities in the male rat. In the case of trans-decalin, the metabolite trans,trans-1-decalol was found in the urine of male rats and not in the female rat urine. Also, the metabolite trans,cis-2-decalol was found in larger quantities in male rat urine.

TABLE 5. URINARY METABOLITES DETECTED IN MALE AND FEMALE FISCHER 344 RATS TREATED WITH CIS- AND TRANS- DECALIN

	RELATIVE AMOUNT OF METABOLITE DETECTED (GC PEAK AREA)	
	MALE RAT	FEMALE RAT
CIS-DECALIN		
Cis,Cis-2-Decalol	4.0	3.3
Cis,Cis-1-Decalol	1.0	ND[a]
Cis,Trans-1-Decalol	2.6	1.0
TRANS-DECALIN		
Trans,Cis-2-Decalol	5.7	1.0
Trans,Trans-1-Decalol	1.0	ND[a]

a = None-detectable

Analysis of kidney extracts from male and female Fischer 344 rats dosed with cis- and trans-decalin also proved interesting. Renal damage was observed in all of the male rats dosed with cis-decalin, and the presence of cis-2-decalone was detected in homogenized kidney extracts (Olson et al., 1985). For the male rate dosed with trans-decalin, 5 of 6 rats showed kidney lesioins and trans-2-decalone was present in their kidney extracts. The single male rat which presented no renal damage following trans-decalin dosing had no detectable trans-2-decalone in its kidney extract. None of the female Fischer 344 rats dosed with cis- or trans-decalin had kidney damage and 2-decalone was absent from their kidney extracts. The presence of ketones in the kidney extracts of male rats exposed to cyclic hydrocarbons suggests that the ketone could be the causative agent of renal damage or a chemical marker indicating the occurrence of renal damage.

TETRALIN

Since the cyclic hydrocarbons JP-10 and cis-and trans-decalin both produced renal lesions in male Fischer 344 rats, an evaluation of other cyclic hydrocarbons was considered necessary. These evaluations were designed to determine if all cyclic hydrocarbons, regardless of structure, are capable of eliciting nephrotoxic effects, or if by altering the hydrocarbons structure, the nephrotoxic effect can be reduced or eliminated. A logical cyclic hydrocarbon to study for rat nephrotoxic effects was tetralin (tetrahydronaphthalene) (See figure 4). Tetralin, like decalin, contains ten carbons and is composed of two fused six-membered rings. However, the structural and electronic character of tetralin is unlike decalin.

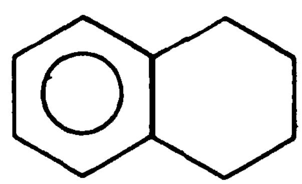


FIGURE 4. TETRALIN

Structurally, the aromatic ring of tetralin causes that part of the molecule to be planar while the aliphatic portion of the molecule remains non-planar. Both of the decalin isomers are composed of two, fused cyclohexane rings which exist in a non-planar chair configuration.

Electronically, the aromatic ring of tetralin will activate the alpha-carbons towards oxidation. The structural and/or electrical differences may preclude or facilitate the metabolism of tetralin to potentially toxic molecules.

A study of urinary metabolites from male and female Fischer 344 rats exposed to tetralin may provide information about positions on the tetralin molecule which undergo oxidation and the effect they have on renal toxicity. Additionally, the presence or absence of tetralin metabolites in kidney extracts, along with histopathology of kidney tissues, should help clarify the function of the metabolites in eliciting renal damage.

PROBLEM STATEMENT A. (Tetralin)

The objectives of this research were to orally does male and female Fischer 344 rats every other day with tetralin over a 14 day period and collect and evaluate the following information:

1. The effects of tetralin dosing on animal weight gain as compared to sham controls dosed with water;
2. Histopathologic evaluation of kidney and liver tissues from exposed animals in order to determine damage and note any differences which appear to be a function of sex;
3. Identification of urinary metabolites of tetralin in dosed Fischer 344 rats and differences in structure and relative amounts between males and females;
4. Identification of tetralin metabolites isolated from the kidneys of tetralin-exposed Fischer 344 rats and any differences between males and females;

5. The effects of cytochrome oxidase system inhibitors and inducers on tetralin metabolism, resultant metabolite production, and the appearance of renal lesions with respect to male and female Fischer 344 rats.

INDAN

The compound indan, or 1,2-dihydroindene is a totally planar molecule containing 9 carbons. An examination of the capability of indan producing the aforementioned nephrotoxic situation would provide insight as to the structural requirements necessary for cyclic hydrocarbons ability to elicit nephropathy. Indan's molecular formula is C_9H_{10} with a molecular weight of 118 (See figure 5). The physical constants are as follows: boiling point, 178°C; melting point,-54°C; density, 0.9639g/ml.

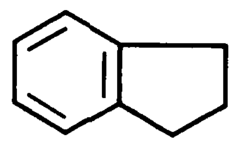


Figure 5. Indan

Earlier metabolism studies involving indan utilized in vitro methods to determine the metabolic pathways. Billings et al. (1971) studied the oxidation of indan by the hepatic microsomal system. They concluded indan was oxidized in two steps to a mixture of 1-indanol and 1-indanone by a rat liver microsomal preparation. The metabolism of indan started first with hydroxylation followed by dehyrogenation to the ketone. However, these metabolites accounted for only 5% of the dose recovered in the corresponding in vivo study. The majority of the metabolism products were unidentified. A follow-up study isolated the enzyme responsible for dehydrogenation of the alcohol and found it to have narrow substrate specifity. Only closely related molecules such as 1-tetralol, and fluorol are oxidized by this enzyme (Brooks, 1956).

Research has also been accomplished on closely related compounds. Brooks and Young (1956) dosed rabbits and rats with indene and discovered indene was converted into cis- and trans-1,2-indandiol and 2-indanone. The metabolism of cis- and trans-1,2-indandiol in rats was investigated by Lewis (1966). The product 2-hydroxy-1-indanone was detected along with the starting materials in the urine. Finally, 1-and 2-indanone metabolism in rabbits was examined. The dihydrodiols was again found as a metabolite along with unspecified products (Balsamo, 1974).

PROBLEM STATEMENT B. (Indan)

The objectives of this research were to orally dose both sexes of Fischer 344 rats every other day with indan over a 14 day period and collect and evaluate the following information.

1. The physiological and histopathlogical effects caused by the indan as compared to controls.
2. Synthesize possible metabolites of indan.
3. Identify the urinary and kidney metabolites in dosed Fischer 344 rats.
4. Evaluate the effects of cytochrome oxidase inhibitors and inducers on Indan metabolism, resultant metabolite production and the appearance of renal lesions with respect to male and female Fischer 344 rats.

II. METHODS

A. Tetralin

MATERIALS

Twelve Fischer 344 male rats approximately 4 months in age and weighing 311 ± 18 g and twelve female rats approximately 4 months in age and weighing 185 ± 6 g were purchased from Charles River Breeding Laboratories, and randomly allocated to exposure groups (see Table 5). From these 24 animals, six male rats and six female rats were given 0.5 mL/kg (485 mg/kg) body weight neat tetralin intragastrically on alternate days over a 14 day period. Equal numbers of control rats for both sexes were given 0.5 mL/kg body weight of water intragastrically. Following exposure to tetralin, the rats were placed in metabolism cages for 24 and 48 hour urine collection, after which they were housed in plastic cages. All rats were weighed daily. Water and feed (Ralston Purina Co., St. Louis, MO) were available *ad libitum*. At the end of the 14 day dosing period, rats were placed in metabolism cages for overnight urine collection and then sacrificed by anesthetic overdose. One kidney and the median lobe of the liver from each rat were harvested for histopathologic evaluation. Tissues were immersed in 10% neutral buffered formalin, imbedded in paraffin blocks, and cut into sections 6 microns in thickness. They were then mounted on glass slides and stained with routine hematoxylin and eosin. The other kidney was used for metabolite analysis. Urine and kidney samples were frozen until metabolite analyses were performed.

ANALYTICAL PROCEDURES

Isolation of tetralin metabolites from urine and kidney samples was accomplished using the extraction technique of Yu (1985). Urine collected from study animals was allowed to thaw at room temperature. Equal volume aliquots from the same urine sample were adjusted to a pH of 4.0. A 0.5 mL volume of glucuronidase/sulfatase (Calbiochem, La Jolla, CA) was added to one aliquot of each sample which was then heated to 37°C with shaking for 16 hours. Following incubation, aliquots were cooled to room temperature, and then filtered separately through Clin-Elut tubes (Analytichem International, Harbor City, CA) using methylene chloride as eluent. The kidneys saved and frozen for metabolite analysis were allowed to thaw, homogenized in distilled water, and processed using the same procedure as for the urine. The eluates from both the urine and kidney samples were individually concentrated by evaporation under nitrogen in preparation for gas chromatographic analysis.

Gas chromatography was used to analyze the metabolites and extracted components from the urine and kidney samples. A Hewlett-Packard 5880A gas chromatograph equipped with a flame ionization detector was used. A 10 m x 0.2 mm I.D. carbowax 20M fused silica capillary column (Hewlett-Packard, Palo Alto, CA) provided good separation for urinary extracts and a 15 m x 0.25 mm I.D. carbowax 20M fused silica capillary column (Supelco, Bellefonte, PA) was used for analysis of kidney extracts. For analysis of urine extracts, oven temperature was programmed from 60° to 170°C at 5°/min after an initial delay of one minute and a hold at final temperature for 30 minutes. A temperature program from 100° to 190°C was used for kidney extracts with a final holding time of 35 minutes. For both urine and kidney analyses, detector and injection port temperatures were 250°C and 200°C, respectively. Helium was used as the carrier gas with a split ratio of 20:1. The linear velocity was 23.5 cm/sec at 100°C for the 10 meter column and 34.2 cm/sec at 100°C for the 15 meter column.

Metabolite identification was accomplished using a Hewlett-Packard gas chromatograph/mass spectrometer (GC/MS). The mass spectrometer used was a quadrupole instrument and ionization was obtained by electron impact at a voltage of 70 eV with an ion source temperature of 200°c. Helium was used as the carrier gas with an injection port temperature of 200°C. For urine extract analysis, the same 10 m x 0.2 mm I.D. carbowax 20M fused silica capillary column was used with a linear velocity of 21 cm/sec at 100°C. Oven temperature was held at 60°C for one minute and then programmed at 5°/min to 170°C with a final holding time of 30 minutes. A 20 m x 0.25 mm I.D. carbowax 20M fused silica capillary column (Supelco, Bellefonte, PA) was used for kidney extract analysis with a linear velocity of 32.5 cm/sec at 100°C. Column temperature was programmed from 100° to 190°C at 5°/min with initial hold of one minute and final time of 30 minutes. Comparison of mass spectra fragmentation patterns with the fragmentation patterns from purchased or synthesized compounds confirmed identification of urinary and kidney metabolites.

CHEMICALS USED FOR METABOLITE IDENTIFICATION

In order to identify tetralin metabolites in urine and kidney extracts, chemicals were purchased from the Aldrich Chemical Co., Milwaukee, Wisconsin. Chemicals directly used for analysis were: tetralin, 1-tetralol, 1-tetralone, 2-tetralone, 5,6,7,8-tetrahydro-1-naphthol, 5,6,7,8-tetrahydro-2-naphthol. The following chemicals were used in the synthesis of possible tetralin metabolites: tetraethylammonium hydroxide, t-butylhydroperoxide, osmium tetroxide, 1,2-dihydronaphthalene, m-chloroperoxybenzoic acid, trimethylsilyl chloride, triethylamine, chromyl chloride, 1,4-naphthoquinone, and lithium aluminum hydride.

SYNTHETIC PREPARATIONS

2-Tetralol

A mixture of lithium aluminum hydride (1.9g, 0.05 mole) in 200 mL of ether was added to a solution of 1-tetralone (15g, 0.1 mole) in 50 mL of ether. The solution was refluxed for 4 hours. After cooling, 50 mL of water was added dropwise with stirring. The ether solution was decanted off and dried over anhydrous sodium sulfate. Removal of the ether left a solid which when recrystallized from petroleum ether yielded 2-tetralol (10.8 g, 0.074 mole, 74% yield), mp 50-1^{o}C (Lit mp 50-1^{o}C) (Pickard, 1912).

1,2,3,4-Tetrahydronaphthalene-cis-1,2-diol

A 250 Erlenmeyer flask was charged with 100 mL of t-butyl alcohol, 7.5 mL of 10% aqueous tetraethylammonium hydroxide and 1,2-dihydronaphthalene (5g, 0.022 mole). After cooling to 0^{o}C in an ice bath, 9 mL of 90% t-butylhydroperoxide and 5 mL of 0.5% osmium tetroxide in t-butylalcohol were added. The resulting solution was stirred for 2 hours at 0^{o}C and placed in a refrigerator overnight. After 50mL of 5% sodium bisulfite was added, the solution was allowed to warm to room temperature with stirring. Removal of the t-butyl alcohol under reduced pressure yielded a residue which was extracted with ether. The ether solution was passed through a 10 cm column of neutral alumina. Evaporation of the ether gave 1,2,3,4-tetrahydronaphthalene-cis-1,2-diol (2.1 g, 0.013 mole, 58% yield), mp 100-2^{o}C (Lit mp 102^{o}C) (Strauss, 1921).

<u>1,2,3,4-Tetrahydronaphthalene-trans-1,2-diol</u>

A solution of 85% m-chloroperoxybenzoic acid (24 g, 0.115 mole) and 200 mL of methylene chloride was cooled to 0^{o}C in a 500 mL Erlenmeyer flask. 1,2-Dihydronaphthalene (10 g, 0.08 mole) in 25 mL of methylene chloride was added dropwise. The solution was allowed to warm to room temperature and then was stirred for 12 hours. The solution was cooled to 0^{o}C and the precipitated m-chlorobenzoic acid was filtered off. The filtrate was washed consecutively with 25 mL of water, 2-50 mL portions of 10% sodium hydroxide, and 25 mL of water. After drying over sodium sulfate, the methylene chloride was removed under reduced pressure. The 3,4-dihydronaphthalene-1,2-oxide (6.3 g, 0.043 mole, 53% yield) was distilled at 120-2^{o}C (15 Torr), (Lit bp 124-5^{o}C) (13 Torr) (Strauss, 1921). A mixture of 3,4-dihydronaphthalene-1,2-oxide (4 g, 0.027 mole) in 10 mL of ether was added dropwise to a solution of lithium aluminum hydride (1 g, 0.037 mole) and 100 mL of ether. The solution was refluxed for 4 hours. Upon cooling, the solution was hydrolyzed. Separation and drying of the ether layer yielded 1,2,3,4-tetrahydronaphthalene-trans-1,2-diol (3.8 g, 0.023 mole, 86% yield), mp 110-2°C (Lit mp 112°C) (Strauss, 1921).

2-Hydroxy-1-tetralone

1-tetralone (36 g, 0.25 mole) was added to a solution of trimethylsilyl chloride (32.6 g, 0.30 mole) and triethylamine (60.6 g, 0.60 mole) in 100 mL of of dimethylformamide. The solution was refluxed for 48 hours. Upon cooling, the solution was diluted with 200 mL of pentane. After washing with 2-300 mL portions of cold 5% sodium bicarbonate solution, the solution was dried over sodium sulfate. Distillation yielded 1-trimethylsilyloxy-3,4-dihydronaphthalene (47 g, 0.22 mole, 76% yield), bp 90-3°C (30 Torr), (Lit bp 78-9°C) (17 Torr) (House, 1969). Chromyl chloride (2 g, 0.012 mole) in 10 mL of methylene chloride was added to a stirred solution of 1-trimethylsilyloxy-3,4-dihydronaph- thalene (2.2 g, 0.01 mole) and 20 mL of dry methylene chloride under nitrogen at -78°C. After stirring for 30 minutes at -78°C, the solution was added to a 25 mL cold 5% sodium bisulfite solution and stirred for 15 minutes. The resulting green solution was neutralized with a 5% sodium bicarbonate solution. After filtration through a Buchner funnel, the solution was extracted with 2-100 mL portions of methylene chloride. The solution was then chromatographed on an alumina column to give 2-hydroxy-1-tetralone (1.3 g, 0.008 mole, 67% yield), mp 58-60°C (lit mp 58-60°C) (Vedejs, 1978).

4-Hydroxy-1-tetralone

1,4-naphthoquinone (3.0 g, 0.019 mole) was reduced with lithium aluminum hydride (1.0 g, 0.027 mole) in 250 mL of ether using a Soxhlet extractor. The hydride solution was then hydrolyzed. The ether layer was separated and immediately subjected to chromatography on an alumina column. Evaporation of the ether yielded a viscous oil which showed hydroxyl and ketone peaks in the infrared spectrum. Mass spectrometry showed a molecular ion m/z = 162 (Boyland, 1951). By-products of the reaction were 1,2,3,4-tetrahydronaphthalene-cis- and trans-1,4-diols.

To confirm the presence of hydroxy-ketones, the urine from three male rats exposed to tetralin was pooled and added to 2 mL of methylene chloride. The solution was then reduced using a mixture of lithium aluminum hydride (1.0 g, 0.026 mole) in 300 ml of ether. Stirring was continued overnight at room temperature. The ether layer was separated and dried over anhydrous sodium sulfate. After evaporation of the ether, the residue was taken up in methylene chloride and analyzed by GC/MS.

RESULTS AND DISCUSSION

HISTOPATHOLOGY

Only male rats exposed to tetralin exhibited recognizable renal lesions. When compared with controls where minimal to mild hyaline droplets were observed, exposed males exhibited increased cytoplasmic hyaline droplets in proximal convoluted tubular epithelial cells. In nonpretreated exposed males the severity of hyaline droplet formation was regarded as moderate when hyaline droplet formation was graded on a scale where 0=no droplets, 1=minimal, 2=mild, 3=moderate, and 4=severe. Female control and exposed rats did not display hyaline droplet formation. The morphology of the droplets varied from elongated, crystalline forms to homogeneous spheroids of assorted dimensions. Additionally, foci of cellular degeneration were present within the proximal convoluted tubules of male rats and consisted of tubular segments where epithelial cells exhibited increased cytoplasmic basophilia and vesicular nuclei. Intratubular cellular casts, overt glomerular changes or significant inflammation was not seen.

As discussed in the introduction, several mechanisms may contribute to accentuated hyaline droplet formation and cellular degeneration within proximal convoluted tubules. Since findings suggest that alpha 2u globulin is the major constituent of hyaline droplets, the interaction of hydrocarbons or their metabolites with indigenous proteins like alpha 2u globulin or lysosomal enzymes might compromise protein catabolic pathways. Therefore, mechanisms involving hydrocarbons and their metabolites that cause this excessive protein accumulation within proximal tubular epithelial cells could be basic to the pathogenesis of hydrocarbon-induced nephropathy.

METABOLITE ANALYSIS

Possible alcohol and ketone metabolites were either purchased or synthesized and then analyzed by gas chromatography/mass spectrometry. To determine if detector response to these compounds was uniform, various known concentrations of each metabolite were analyzed by gas chromatography and the detector response, reported in peak area, determined. For each ketone and alcohol metabolite analyzed, the detector yielded a constant area count for a 1 ug sample of the metabolite. Hydroxyketones and diols could not be separated in pure form, and therefore, a precise detector response to these compounds could not be determined. However, after compensating for impurities, it can be estimated with some confidence that detector response to hydroxyketones and diols during urine and kidney extract analysis was also uniform.

METABOLITES OF TETRALIN

Six tetralin metabolites were identified in the urine of male and female tetralin exposed rats. These were: 1-tetralol, 2-tetralol, 2-hydroxy-1-tetralone, 4-hydroxy-1-tetralone, 1,2-tetralindiol, and 1,4-tetralindiol. It is important to note that the urinary metabolites recovered following tetralin exposure were primarily disubstituted molecules whereas the metabolites identified from exposure to other cyclic hydrocarbons such as decalin and JP-10 have been monosubstituted molecules. Representative gas chromatographic tracings of urine samples from male rats treated with tetralin and from control rats are shown in Figure 5 and Figure 6, respectively. Mass spectra of synthesized tetralin metabolites are presented in Figure 7. Trace quantities of naphthol were also detected in male and female urine samples but were considered to be the metabolite of the minor naphthalene impurity present in the tetralin used for dosing.

FIGURE 7. GAS CHROMATOGRAPHIC TRACING OF URINE FROM MALE RATS DOSED WITH TETRALIN. TRACING IS REPRESENTATIVE OF MALE AND FEMALE RATS.

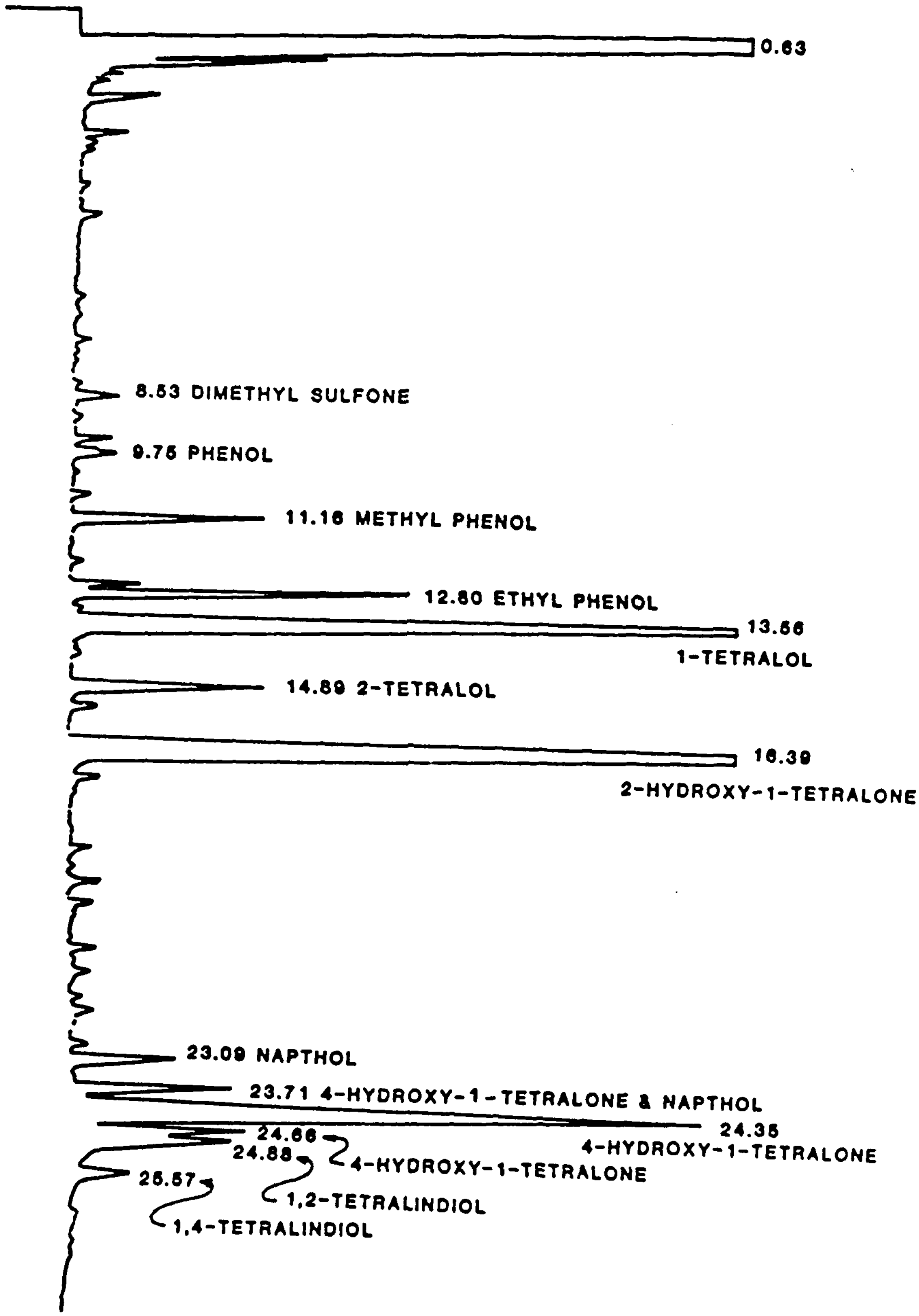


FIGURE 8. GAS CHROMATOGRAPHIC TRACING OF URINE FROM MALE RATS DOSED WITH WATER. TRACING IS REPRESENTATIVE OF BOTH MALE AND FEMALE RATS.

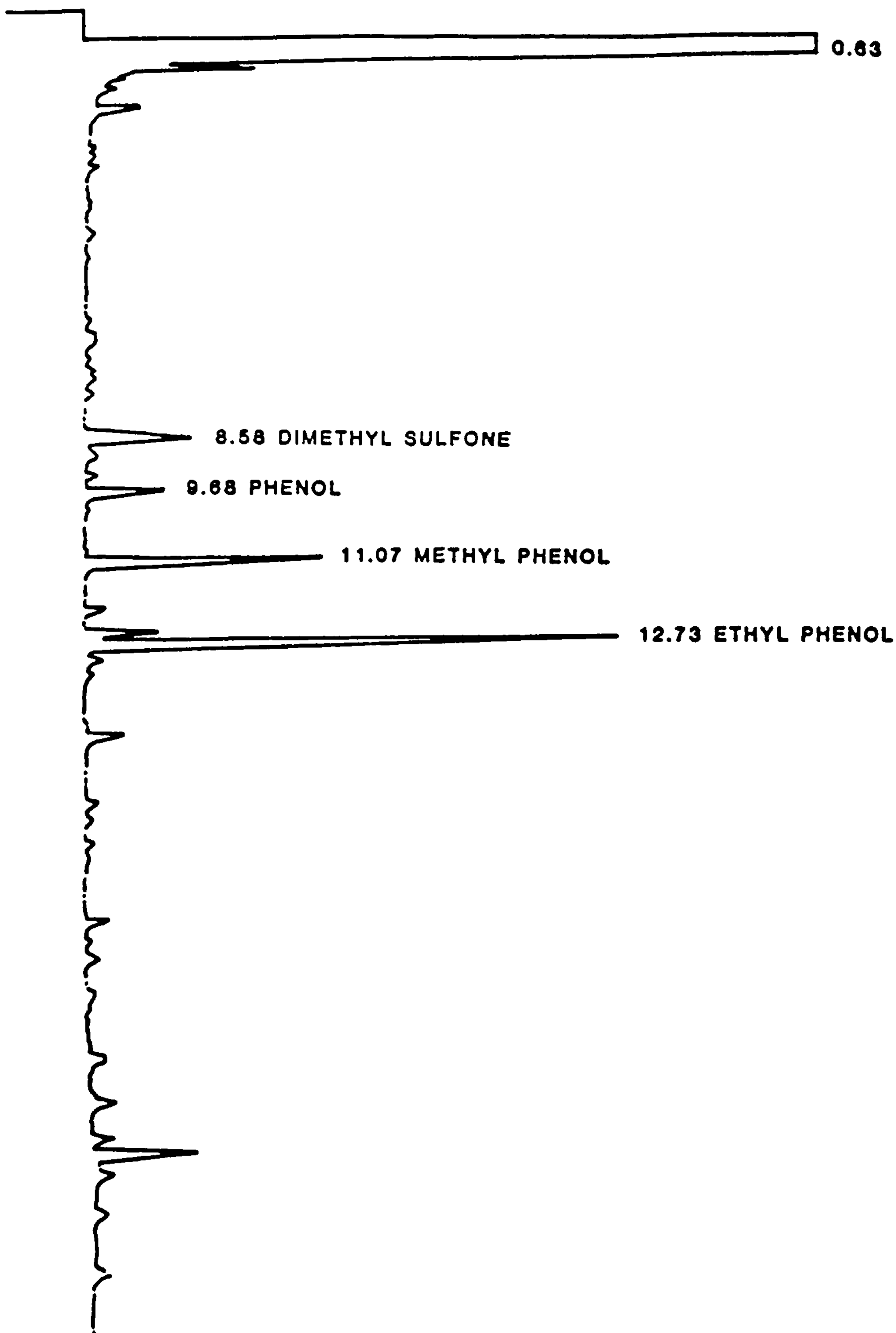
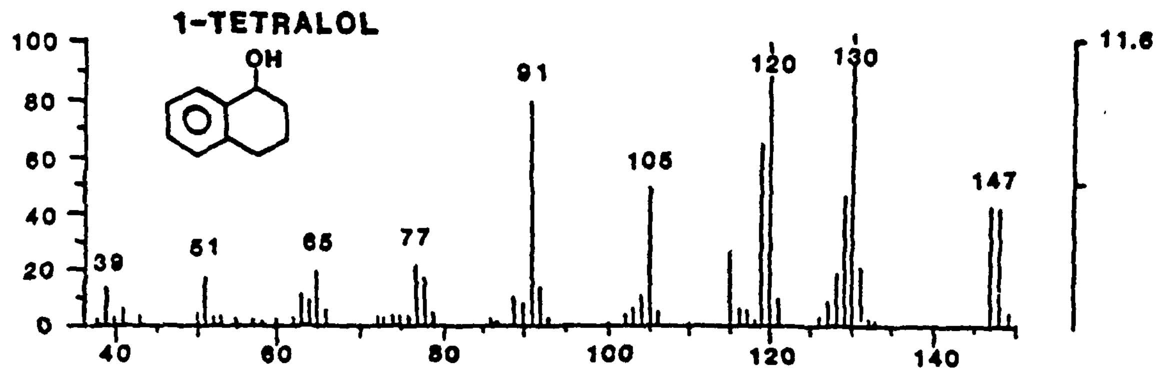
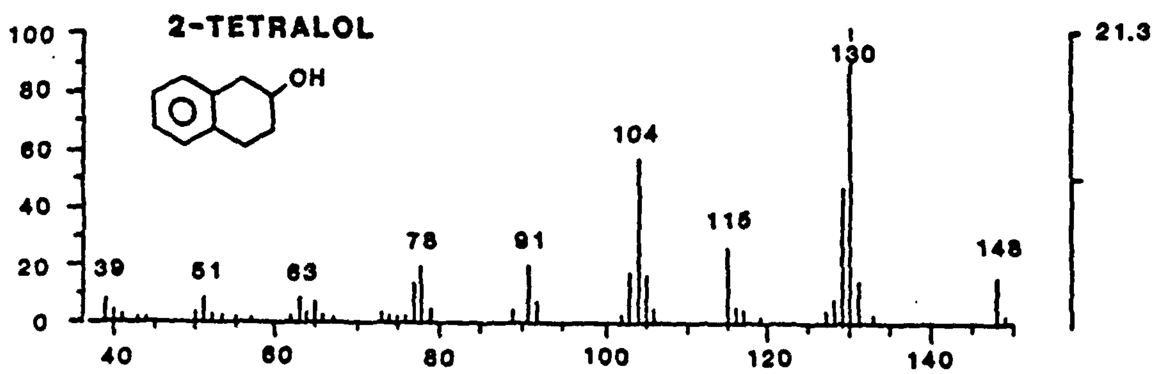
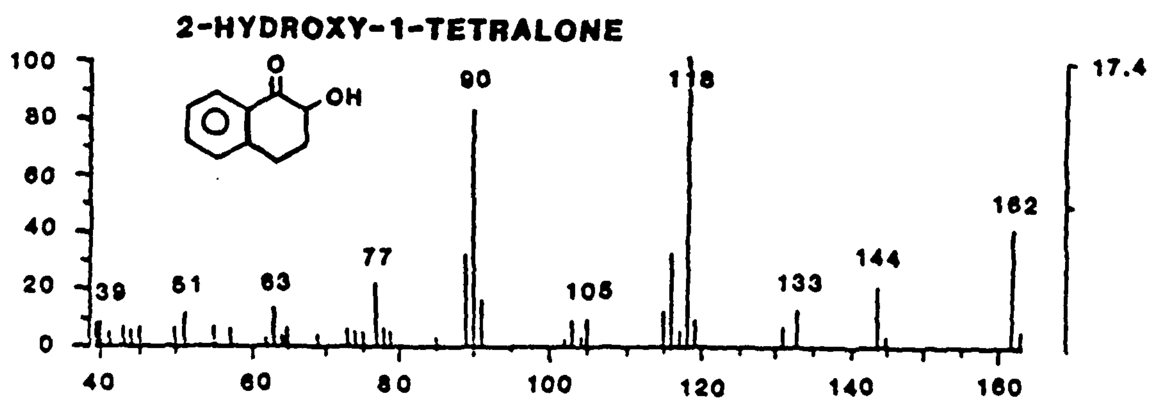


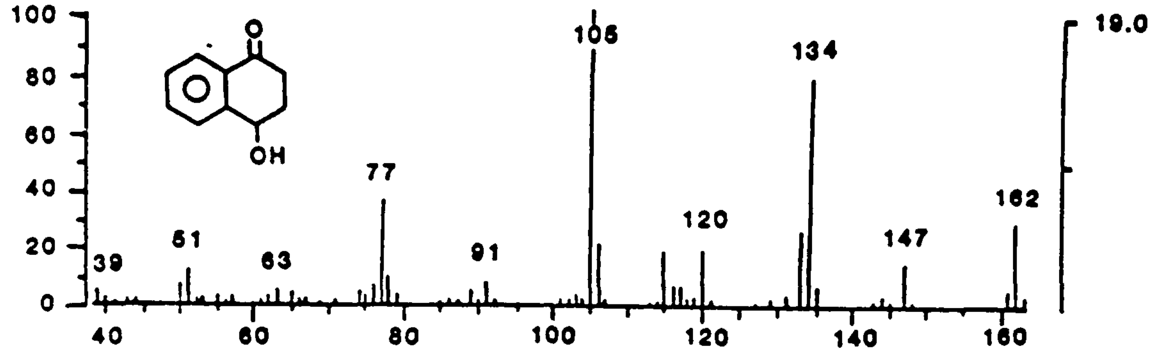
FIGURE 9. MASS SPECTRA OF SYNTHESIZED TETRALIN METABOLITES



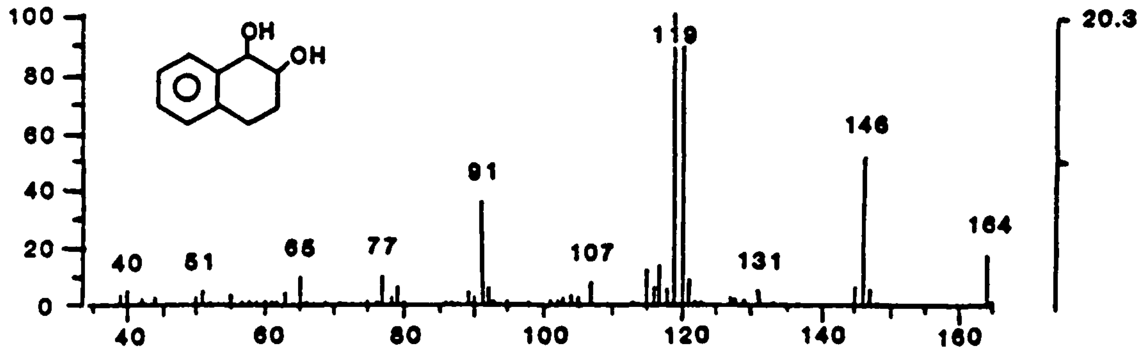




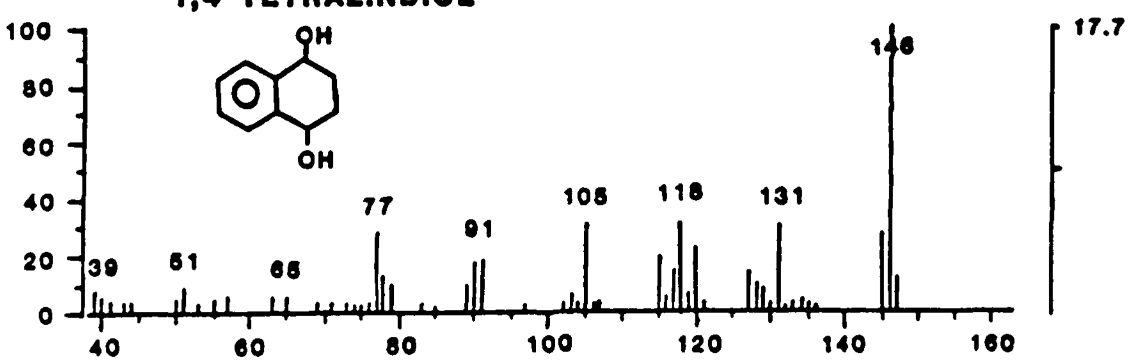
4-HYDROXY-1-TETRALONE



1,2 -TETRALINDIOL



1,4-TETRALINDIOL



Relative amount percentages of identified metabolites for male and female rats dosed with tetralin are shown in Table 8. Because of inconsistent resolution during gas chromatographic detection, relative amount comparisons could not be accurately determined for 1,2-tetralindiol in male and female rats and for 1,4-tetralindiol and final 14th day urine amounts of 4-hydroxy-1-tetralone in female rats. Also, three peaks were identified by GC/MS that contained 4-hydroxy-1-tetralone. The largest peak was pure 4-hydroxy-1-tetralone with the other two peaks containing small amounts of 4-hydroxy-1-tetralone a naphthol and 1,4-tetralindiol. The major 4-hydroxy-1-tetralone peak was used for relative amount comparisons.

The major tetralin metabolites in urine for both male and female rates were 1-tetralol, 2-hydroxy-1-tetralone, and 4-hydroxy-1-tetralone. The relative amount percentages of metabolite recovered for male and female rats were statistically compared for each collection period. Values were considered significantly different at $p \leq .05$ using the Student's t-test. In each case where there was a significant difference, a greater percentage of the metabolite was present in the female rat urine. Relative amounts of 2-tetralol were significantly greater in female rats for 24 hr, 48 hr and final 14th day urine collections. The relative amount of 2-hydroxy-1-tetralone was significantly greater in female rats for 24 hr and final 14th day collections while 1-tetralone was greater for only the final collection period.

TABLE 8. RELATIVE AMOUNT COMPARISONS OF TETRALIN METABOLITES IDENTIFIED IN URINE OF MALE AND FEMALE RATS EXPOSED TO TETRALIN

RELATIVE AMOUNT OF METABOLITE RECOVERED PER COLLECTION PERIOD (Percentage) [a,b]

METABOLITES	24 HR MALE	24 HR FEMALE	48 HR MALE	48 HR FEMALE	FINAL[c] MALE	FINAL[c] FEMALE
1-Tetralol	24 ± 3 (6)	27 ± 3 (6)	27 ± 6 (6)	34 ± 3 (6)	26 ± 2 (6)[e]	37 ± 7 (6)[e]
2-Tetralol	4 ± 1 (6)[f]	8 ± 1 (6)[f]	4 ±.5 (6)[g]	8 ± 1 (6)[g]	4 ±.3 (6)[h]	14 ± 4 (6)[h]
2-Hydroxy-1-tetralone	29 ± 4 (6)[i]	35 ± 2 (6)[i]	33 ± 5 (6)	31 ± 2 (6)	30 ± 2 (6)[j]	42 ± 9 (6)[j]
4-Hydroxy-1-tetralone	33 ± 4 (6)	30 ± 5 (6)	27 ± 2 (6)	28 ± 4 (6)	32 ± 4 (6)	.[d]
1,4-tetralindiol	1 ±.5 (6)	.[d]	2 ±.3 (6)	.[d]	2 ± 1 (6)	.[d]
1,2-tetralindiol	.[d]	.[d]	.[d]	.[d]	.[d]	.[d]

a = Mean ± S.D. (N)
b = Percentages are representative of the relative amount of each metabolite as compared to total area of metabolites listed
c = Overnight urine collections following last dosing
d = Detectable, but unable to resolve and quantitate
e, f, g, h, i, j = The relative amount percentages of metabolite recovered for male and female rats were compared for each collection period. Values with identical superscripts were significantly different from each other a $p \leq .05$ using the Student's t-test.

Tetralin metabolites were not detected during GC/MS analyses of kidney extracts from tetralin-exposed male and female rats. The presence of renal lesions in the kidneys of male rats exposed to tetralin with the absence of tetralin metabolites in the kidney extracts is not in agreement with findings from previous cyclic hydrocarbon studies. In studies where male and female rats were dosed with decalin or JP-10, male rats that exhibited renal damage also had ketone metabolites present in their kidney extracts. However, female rats did not exhibit renal damage and metabolites were not detected in their kidney extracts. In tetralin-dosed female rats, the lack of both renal damage and kidney extract metabolites is in agreement with previous hydrocarbon studies.

II. <u>METHODS</u>

B. Indan

MATERIALS

Eighteen Fischer 344 male rats and eighteen Fischer 344 female rats were purchased from the Charles River Breeding Laboratories and allocated to either exposure groups or control groups. Nine male rats weighing 270 +/- 13g were exposed by gavage with a dose of indan of 0.25 ml/kg body weight with indan. The other nine male rats were dosed with water in the same manner for use as controls. Feed and water were available ad libitum. Animals were weighed daily. Rats were placed in metabolism cages for the first 48 hours following the initial dosing for 24 and 48 hour urine collection. At the end of the exposure period, the rats were placed in metabolism cages for overnight urine collection and then sacrificed by halothane overdose. One kidney and the median lobe of the liver were sent to the Pathology Branch of Armstrong Aerospace Medical Research Laboratory for histopathologic examination. The other kidney was used for metabolite identification. Blood samples were also taken at sacrifice to check for any abnormalities.

Nine female rats weighing 168 +/- 5 were similarly dosed with indan for fourteen days. A control group of the same number with the average weight being 175 +/- 6 g were given water intragastrically as a control. The same procedures were followed as with the male rats except no blood samples were taken.

The kidney and liver tissues, after being sent to the Pathology Branch of AAMRL, were fixed in 10% formalin solution for 48 hours. They were then embedded in paraffin, cut into sections 3-6u in thickness, and mounted on microslides. Sectins were stained with eosin and hemotoxylin prior histopathologic examination.

ANALYTICAL PROCEDURES

The metabolites from the kidney and urine were isolated and identified using the extraction technique previously reported for tetralin.

USED FOR METABOLITE IDENTIFICATION

The following chemical were purchased for use in the synthesis or used directly for analysis of the metabolites of indan.

1-Indanol, Aldrich Chemical Company

2-Indanol, Aldrich Chemical Company

4-Indanol, Chemical Procurement Laboratories Inc.

5-Indanol, Aldrich Chemical Company

1-Indanone, Lancaster Synthesis Ltd.

2-Indanone, Aldrich Chemical Co.

Indene, Aldrich Chemical Co.

Formic acid, Aldrich Chemical Co.

Hydrogen peroxide, Aldrich Chemical Co.

4-Chromanone, Aldrich Chemical Co.

Dihydrocoumarin, Aldrich Chemical Co.

5-Methoxy-1-indanone, Aldrich Chemical Co.

Hexamethyldisilazane, Aldrich Chemical Co.

Trimethylsilyl iodide, Aldrich Chemical Co.

N-Bromosuccinimide, Aldrich Chemical Co.

N-Methylmorpholine-N-oxide, Aldrich Chemical Co.

p-Methoxy-3-phenylpropionic acid, Aldrich Chemical Co.

Aluminum Bromide, Aldrich Chemical Co.

SYNTHETIC PREPARATIONS

cis-and trans-Indan-1,2-diol

Cis- and trans-indan-1,2-diol were prepared according to Balsamo, Berti et al (1937). A mixture of 83% formic acid (80 ml), water (5 ml), and 30% hydrogen peroxide (14 ml) was heated for fifteen minutes at 35°C. Indene (11.6 g, 100 mmol) was then added slowly while the temperature was kept at 35-40°C. Stirring was continued for one hour at 35°C. The reaction was allowed to cool to room temperature where it was stirred overnight. A 50 ml of 6N sodium hydroxide was then added and the mixture heated to 90°C for three hours. The solution, after cooling, was saturated with sodium chloride and extracted with six 30 ml portions of ether. Evaporation under reduced pressure yielded off-white crystals. GC-MS analysis revealed a ratio of cis:trans of 80:20. Yield was 33.4%.

The two isomers were isolated by exploiting the sparing solubility of the transindandiol in $CHCl_3$. The mixture of the isomers extracted using the soxlet method with choroform as the solvent. Upon cooling slightly, the trans diol precipitated and was filtered from the solution. M.P.: 154-6°C; literature value: 158-159°C. The cis diol, left after evaporation of the solvent, was recrystallized from boiling cyclohexane (Brooks and Young, 1956). M.p. : 102-4°C; literature value: 98-99°C.

<u>7-Hydroxy-l-indanone</u>

This compound was prepared according to Louden and Razdan (1954). A mixture of 4-chromanone (4.42 g, 29.9 mmol) and anhydrous aluminum chloride (8.32 g, 62.4 mmol) was fused at 180-190°C for 20 minutes and then heated to 200°C. After cooling, it was treated with an ice-hydrochloric acid solution. The product was extracted with ether and the extract washed with aqueous sodium carbonate and dilute sodium hydroxide. The solution was acididfied with hydrochloric acid and extracted with ether again. The product, 7-hydroxy-l-indanone was recovered as 108-110°C; literature value was 111°C. GC/MS analysis revealed pure 7-hydroxy-l-indanone. The yield was approximately 30%.

<u>4-Hydroxy-l-indanone</u>

Dihydrocoumarin (4.48 g, 30.3 mmol) and anhydrous aluminum chloride (8.12 g, 60.9 mmol) was heated to 170-180°C for three hours by the method according the Louden (1954). The mixture was cooled and treated with a solution of ice and hydrochloric acid. The product was extracted with ether, washed first with aqueous sodium carbonate, and then with dilute sodium hydroxide. After being acidified with hydrochloric acid, the mixture was extracted with ether again. The ether was evaporated leaving off-white cyrstals, m.p. 235°C. Literature value: 239-240°C. GC/MS confirmed the presence of the product, 4-hydroxy-l-indanone. The estimated yield from GC; 45%.

5-Hydroxy-1-indanone

5-Methoxy-1-indanone (3.01 g, 18.6 mmol) and anhydrous aluminum bromide (15.72 g, 59.1 mmol) were placed in a 250 ml round-bottom flask with 100 ml of dry, thiopene-free benzene (U.S. patent 2820813, 1958). After refluxing for 4.5 hours, the mixture was cooled and decomposed with solution of ice and hydrochloric acid. The benzene layer was discarded. The acid layer was extracted with three 20 ml portions of ether. The ether was evaporated to yield orange cyrstals. Recrystallization was accomplished in ethanol. Melting point was 176-178^{o}C, compared to a literature value of 182^{o}C. A direct-insertion probe in the MS showed a parent ion m/e of 148. The yie'd was 13%.

6-Hydroxy-1-indanone

Equal weights of p-methoxy-3-phenylpropionic acid (10.0 g, 56mmol) and thionyl chloride (10.0 g, 84mmol) were warmed gently in a Erlenmeyer flask until the violent evolution of hydrochloric acid and sulfur dioxide ceased. The excess thionyl chloride was removed by heating to 100^{o}C under a vacuum. The product, the acid chloride, was distilled under reduced pressure. (Ingold and Piggott, 1923).

The fusion to form the ring structure accomplished according to House and Larson (1968). The acid chloride was then dissolved in 40 ml of methlyene chloride. Aluminum chloride (6.2 g, 45mmol) in 40 ml of 0^{o}C methylene chloride was dropwise and the mixture was stirred for 4 hours at room temperature. The crude product, 6-methoxy-1-indanone, was chromatographed with ether-hexane. The solvent was evaporated to give tan crystals.

The ether function was cleaved to give the 6-hydroxy-1-indanone following the same procedure as in the synthesis of 5-hydroxy-1-indanone. The yield was 13%. The literature value for the compound was 151-153°C, compared to 147-148°C found experimentally.

<u>2-Hydroxy-1-indanone</u>

A dry 1000 ml round bottom flask was charged with 1-indanone (5.31 g, 40mmol), 600 ml of pentane and hexamethyldisilazane (10.1ml, 48mmol). The mixture was cooled to -20°C using a slush bath of liquid nitrogen and carbon tetrachloride. Trimethylsilyl iodide (6.3ml, 45mmol) was added dropwise and the mixture was stirred for 10 minutes at approximately -10°C. After warming to room temperature, the solution was stirred for an additional 2 hours. The slurry was filtered and the pentane solution analyzed by GC/MS and shown to contain the trimethylsilyl enol ether derivative. This procedure was reported by Miller and McKean (1979).

The solvent, pentane, was removed by distillation under reduced pressure. The oxidation of the silyl enol ether was accomplished using the method employed by McCormick et al. (1981). A solution containing the crude silyl enol ether in 25.5 ml of t-butyl alcohol was added at -5°C to a solution containing 3.4 ml of 2.5%(w/v) osmium tetraoxide (0.34mmol), 1.7 ml pyridine, 1.7 ml distilled water, N-methylmorpholine-N-oxide (1.81 g, 15.4mmol), and 57 ml of t-butyl alcohol. This slurry was stirred at 0°C for 3 hours and after warming to room temperature, it was stirred for an additional 6 hours. Osmium tetroxide was removed by adding sodium hydrosulfite (2.98 g) and florisil (11.39 g) and filtering. The filtrate was neutralized to pH of 7 and the t-butyl alcohol was removed under reduced pressure. After further acidification to a pH of 2 and saturation with sodium chloride, the mixture was extracted with ethyl acetate. The ethyl acetate was evaporated to give a brown oil. GC/MS analysis showed the presence of 2-hydroxy-1-indanone. The yield estimated by GC/MS was 5%.

3-Hydroxy-1-indanone

1-Indanone (10.6 g, 80mmol) was dissolved in carbon tetrachloride with N-bromosuccinimide (6.9 g, 40mmol) and a trace of benzoyl peroxide. The mixture was heated under reflux overnight and then cooled to $0^{o}C$. After filtration, the solvent was evaporated, leaving 3-bromo-1-indanone. The 3-hydroxy-1-indanone was prepared according to Undheim and Nilsen (1975). The 3-bromo-1-indanone was put into solution with 400 ml of distilled water and 200 ml of acetone. The acetone was evaporated and more water was added. The compound was extracted with chloroform to yield 3-hydroxy-1-indanone, yield was 60%.

III. RESULTS AND DISCUSSION

PHYSIOLOGICAL EFFECTS

Eight male rats, weighing an average of 259 +/- 9 g were initially dosed with the hydrocarbon, indan at a concentration of 0.5 ml/kg body weight. A control group of eight male rats was dosed with water. Seven of the indan dosed rats died during the first 24 hour exposure period while the eighth rat died on the 5th day.

During this exposure period, several observations on the conditions of the exposed animals were made. The urine collected for metabolite analysis from the exposed male rats showed a red discoloration relative to the urine from the control male rats. Following each dosing, the exposed group appeared listless compared to the control group. Additionally, the male rats dosed with the indan lost a significant amount of weight, an average of 45 +/- 9 g. The observed weight loss for indan exposure translated to 18 +/- 5 % of their total body weight in 6 days.

Since the initial dose of indan was too large, a second group of nine new male Fischer 344 rats (270 +/- 13 g) was dosed by gavage with 0.25 ml/kg body weight, one-half the prior dosage, in the same manner previously listed. Six control rats (272 +/- 12 g) were given the same dosage using water. Likewise, eighteen female Fischer 344 rats were utilized for this experiment. Nine females (173 +/- 5 g) were exposed to the indan while the other nine (175 +/- 6g) were used for controls. The urine from both sexes of indan exposed animals showed a red discoloration.

Because of this red discoloration, 24 hour urine samples were sent to Roche Biomedical Laboratories, Inc., for analysis. Coproporphyrin and uroporphyrin were detected which could account for the red coloration. These two porphyrin derivatives are biosynthetic intermediates in the manufacture of heme, the main component of myoglobin and hemoglobin in the blood. Blood tests showed the exposed male rats to be slightly anemic but the results were inconclusive.

After five days, the time period in which the first indan dosed group of male rats expired, the average weight loss for the male rats was 8 +/- 2.5 g (excluding one rat which developed a thrombus and was sacrificed early). At the end of the exposure period, the mean weight for the male rats was 263 +/- 14 g with the average weight loss being 7 +/- 3.2 g. This makes the percentage weight loss 2.6 +/- 1.1% as compared to 18 +/- 5% from the previous indan exposure. The control group of male rats gained 11 +/- 4.8 g, a 4.0 +/- 1.7% body weight gain over this time period.

Analyzing the data, the exposed male rats lost the greatest amount of weight after the initial dose. Subsequently, the weight loss occurred fluctuated, decreasing in weight immediately after each dose and then increasing before the next dose. See figure 8.

The exposed female rats also exhibited weight loss. Following five days, the mean weight loss was 3.6 +/- 1.7 g. When the dosing was terminated after two weeks, the famale rats dosed with indan, weighed an average of 169 +/- 6.4 g. The weight loss was approximately 3.2 +/- 1.9 g with a percentage weight loss of 1.9 +/- 1.2%. The female rats dosed with water gained about 4.6 +/- 2.9 g during the exposure period. See table 5.

Examining the relationship between the exposed male and female rats, one finds some similarities. The weight fluctuation between doses was alike as well as the overall characteristic weight loss. However, the female exhibited less % body weight loss, 1.9 +/- 1.2% compared to 2.6 +/- 1.1% shown by the males.

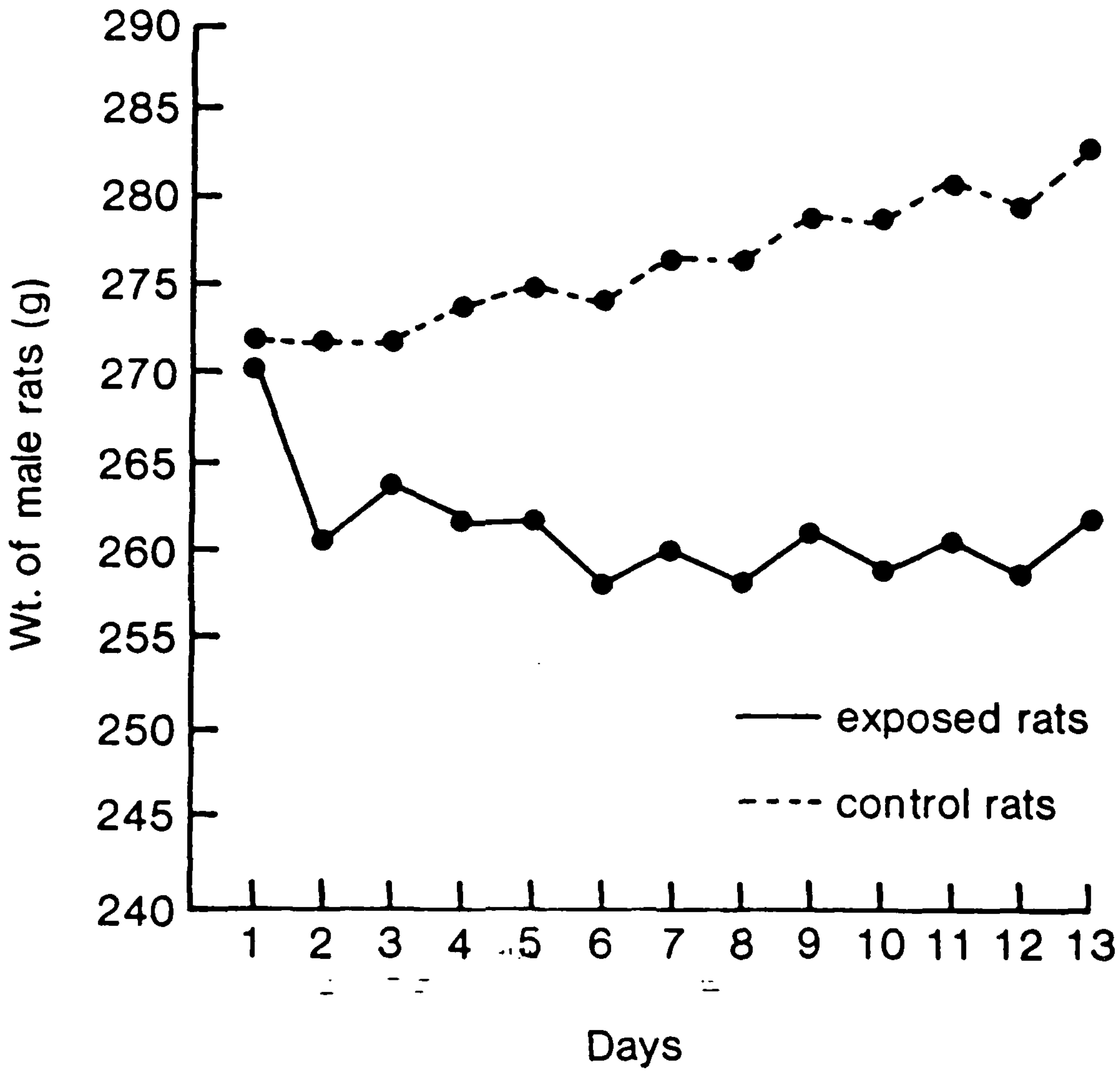


Figure 8 Average weights of experimental animals over test period.

TABLE 5: MEAN WEIGHT ALTERATIONS DURING

14 DAY EXPOSURE PERIOD

GROUP	INITIAL WT. (g)	FINAL WT. (g)	CHANGE (g)
exposed males	270 +/- 13	263 +/- 14	-7 +/- 3.2
control males	272 +/- 12	283 +/- 14	+11 +/- 4.8
exposed females	173 +/- 5	169 +/- 6	-3.6 +/- 1.7
control females	175 +/- 6	179 +/- 5	+4.6 +/- 2.9

HISTOPATHOLOGY

Hyaline droplet accumulation and cellular degeneration found in kidney tissues of male rats are suggestive of nephrotoxicity caused by exposure to hydrocarbons. When compared to controls, male rats exposed to indan showed a mild increase in cytoplasmic hyaline droplets in the epithelial cells of the proximal convoluted tubules of the nephron. Furthermore, some of the droplets appeared as elongated, crystalline forms.

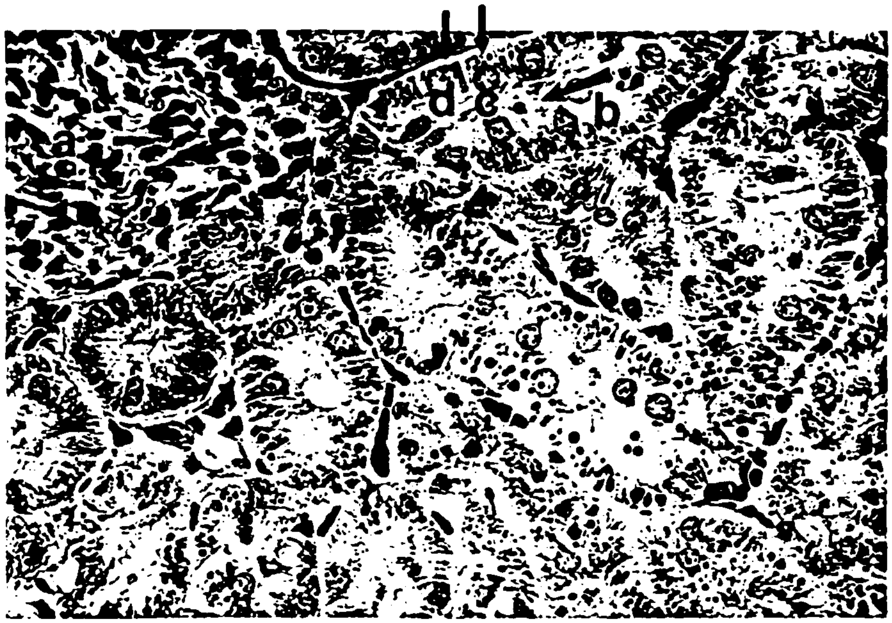


Figure 9: Kidney from exposed male rat. Arrows show a) glomerulus b) proximal tubule c) nucleus of the tubule cell d) hyaline droplet.

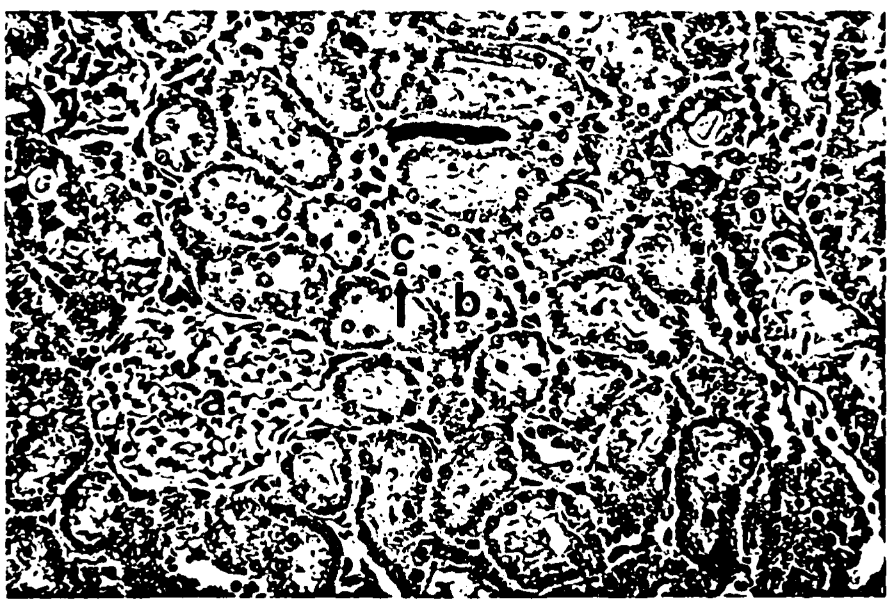


Figure 10: Kidney from control male rat with out hyaline droplet formation. Arrows show a) glomerulus b) proximal tubule cell c) nucleus of the tubule cell.

In discussing the results on the pathologic examinations on the experimental animals, one may assign various degrees of damage in the tissues of the kidney and liver caused by the exposure. In this case, the following scale was used: 0-no effect; 1-minimal; 2-mild; 3-moderate; 4-severe. Using this scale to quantify the cellular degeneration observed in the kidneys, 75 % of the exposed male rats exhibited damage that was rendered a rating of 2.5 or greater (mild to moderate or greater). Only 25% of the male rats used as controls displayed this cellular degradation. This suggests a correlation between renal lesions and exposure to indan. These renal lesions are shown in figure 9 for an exposed animal and compared to the normal control tissue (figure 10).

As previously noted, the main component of these hyaline droplets is the small molecular weight protein, alpha 2u globulin. This protein is filtered through the glomerulus and resorbed back into the body by active transport via epithelial cells of the proximal tubule. Lysosomal enzymes then attack the engulfed protein and break it down into the constituent amino acids which are reused by the body. Somehow, hydrocarbon exposure appears to alter this normal catabolic pathway, either by interacting with the indigenous proteins like alpha 2u globulin, or interfering with the action of the lysosomal enzymes. Finding the specific mechanism could elucidate the exact role hydrocarbons and their metabolism play in nephrotoxicity.

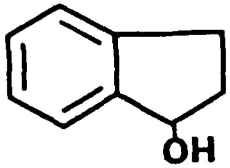
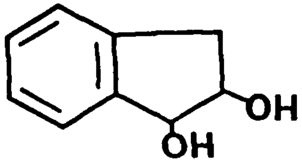
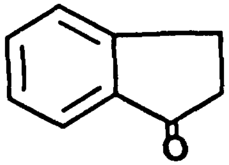
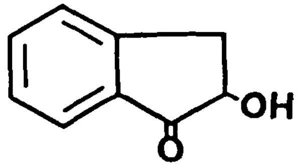
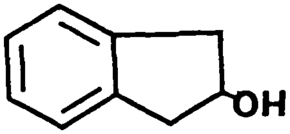
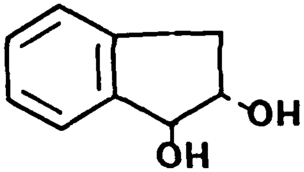
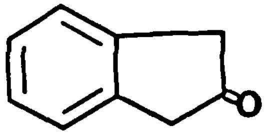
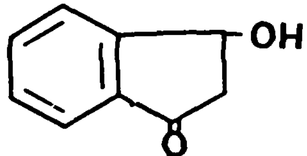
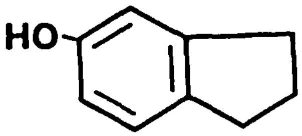
Kidney damage was not seen in the female Indan exposed or control rats. However, some slight alterations were observed in the liver of the exposed females. Seventy seven percent of the females dosed with indan exhibited diffuse hepatocellular cytoplasmic vacuolization that was assigned the rating of 1.0 or greater. In the control females only 22% showed these changes.

METABOLITE ANALYSIS

Characterization of the urinary and kidney metabolites was accomplished through comparison of the GC/MS spectra of urinary and kidney extracts with the spectra of compounds that were bought or independently synthesized. The nine urinary metabolites identified were 1-indanone, 2-indanone, 1-indanol, 2-indanol, 5-indanol, cis-1,2-indandiol, trans-1,2indandiol, 2-hydroxy-1-indanone, and 3-hydroxy-1-indanone. A representative GC tracing of the urinary metabolites of both exposed and control animals is shown in figures 11 and 12, respectively. Table 6 illustrates the structures of the metabolites.

GC detector response was uniform for 1-indanol, 2-indanol, 5-indanol, 1-indanone, 2-indanone, and trans-1,2-indandiol. Detector response to the cis-1,2-indandiol was not linear due to the high concentration of the standard solution that was used. The two hydroxy ketones, which were synthesized at a later date, were assumed to also have a uniform response.

TABLE 6 : URINARY METABOLITES OF INDAN

ALCOHOLS	DIOLS	KETONES	HYDROXY-KETONES
 1-Indanol	 cis-1,2-Indandiol	 1-Indanone	 2-Hydroxy-1-Indanone
 2-Indanol	 trans-1,2-Indandiol	 2-Indanone	 3-Hydroxy-1-Indanone
 5-Indanol			

GAS CHROMATOGRAPHIC TRACING OF URINE FROM MALE RATS DOSED WITH INDAN. TRACING IS REPRESENTATIVE OF MALE AND FEMALE RATS.

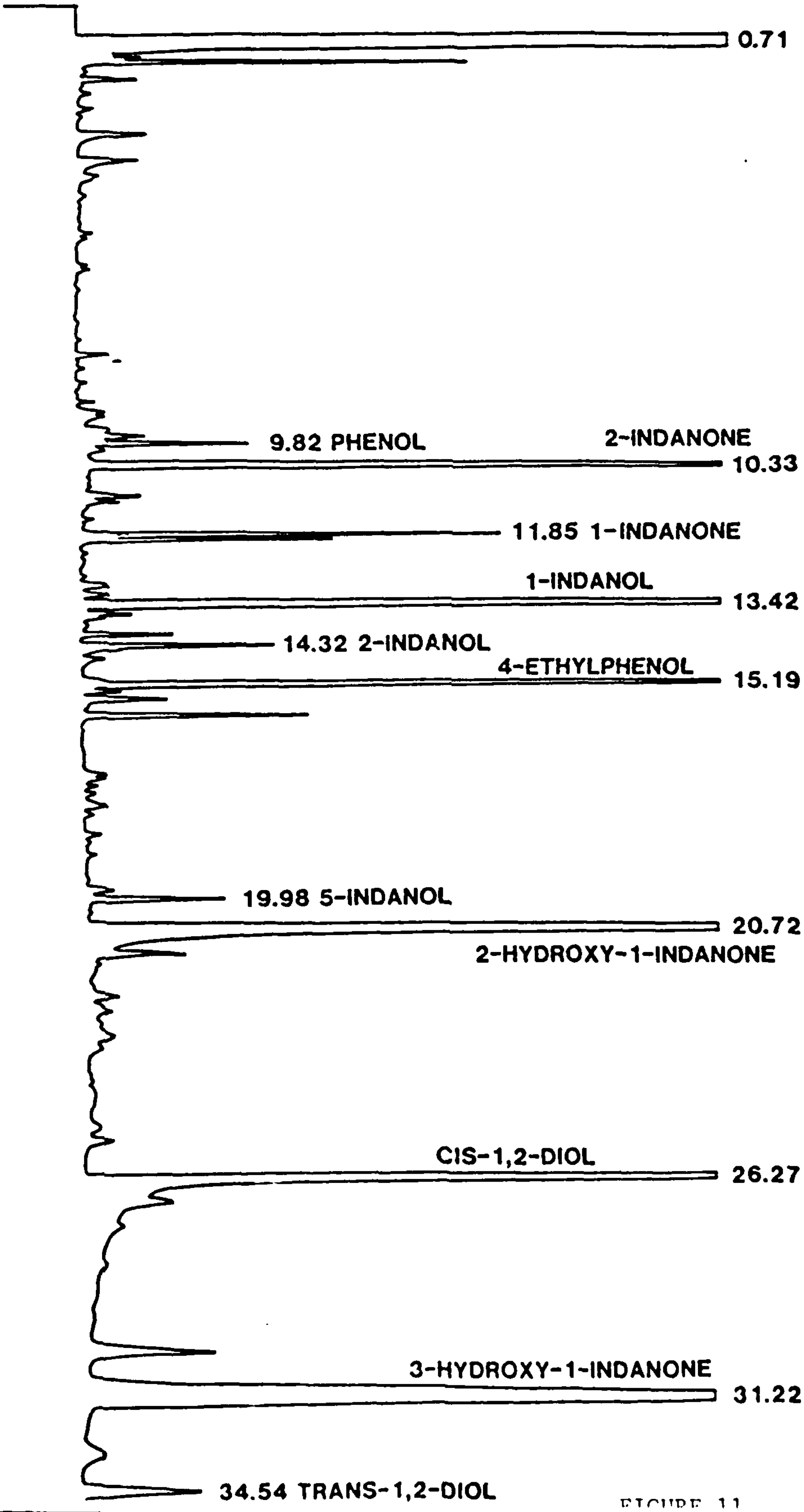


FIGURE 11

GAS CHROMATOGRAPHIC TRACING OF URINE
FROM MALE RATS DOSED WITH WATER.
TRACING IS REPRESENTATIVE OF
BOTH MALE AND FEMALE RATS.

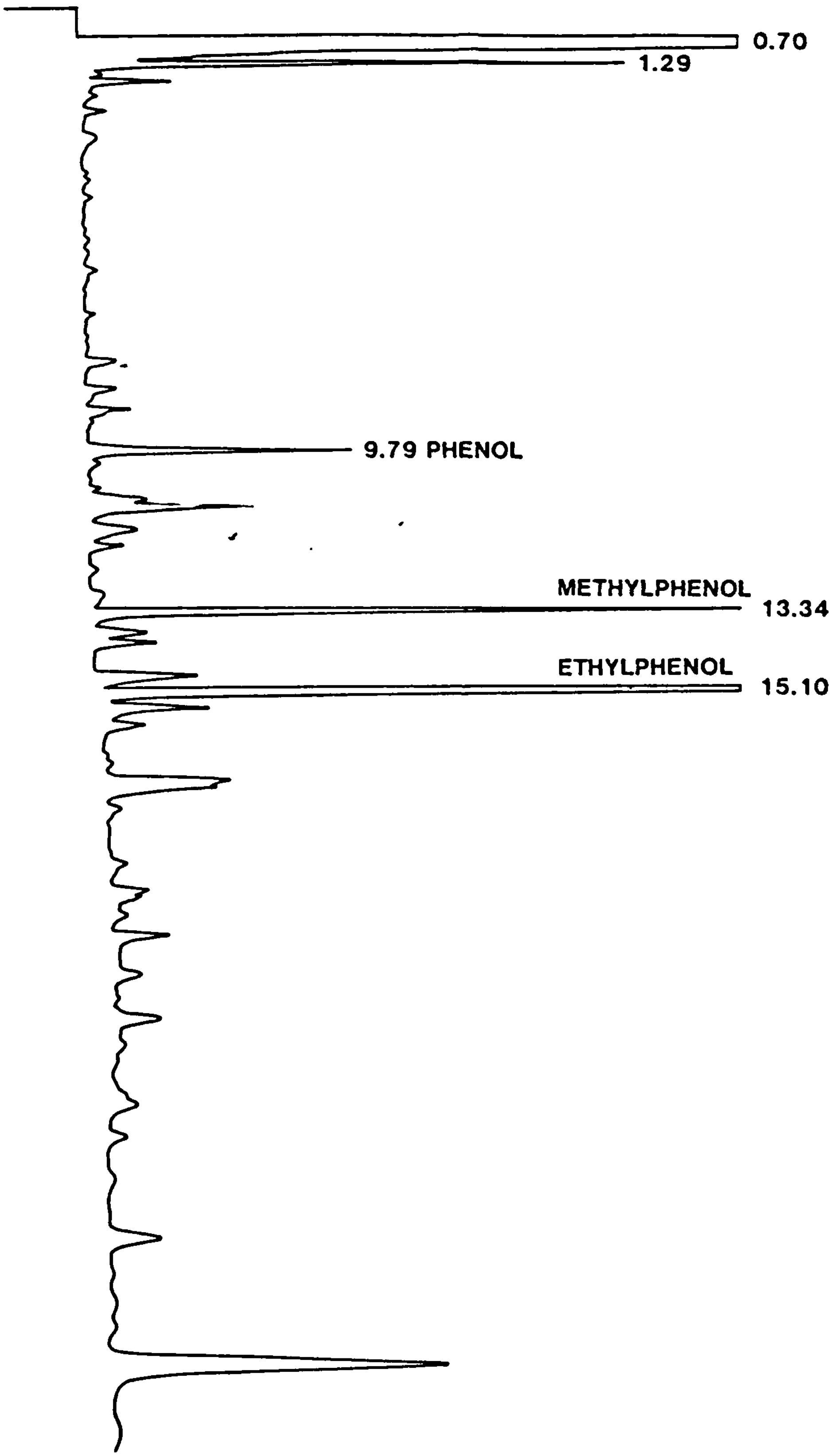


Figure 12

The column used for the GC analysis did not resolve between 1-indanol (R.T.=14.43) and methylphenol (R.T.=14.34). However, the GC/MS instrument was able to separate them and prove the presence of 1-indanol. Comparing the GC area % of each metabolite to total GC area of all the metabolites, the approximate relative amount of each metabolite in the urine was tabulated (table 7).

Only two metabolites were found in the kidney homogenate of male Fischer 344 rats. These were 1-indanol and 1-indanone. No metabolites were isolated from the kidneys of female rats.

Analyzing the results of the relative amounts of each metabolite in the urine several trends can be discerned. The concentration of 5-indanol was fairly constant throughout the dosing period, while the other metabolites varied in concentration. The largest differences noticed for both sexes were the amounts of 2-hydroxy-1-indanone and 3-hydroxy-1-indanone. The concentration of 2-hydroxy-1-indanone was elevated in the 48 hour urine sample relative to the 24 hour sample. The concentration of this metabolite was decreased in the 14th day urine specimen. Conversely, the concentration of 3-hydroxy-1-indanone was the lowest in the 48 hour urine sample and was escalated in the 14th day sample.

Some small variations were observed in the concentrations of the individual metabolites between the male and female rats. In all cases, male rats had slightly less 1-indanone in their urine than the females. However, only kidney homogenates from male rats had 1-indanone present. In addition, the male rats were observed to have a smaller concentrations of the cis- and trans-indandiols in their urine.

TABLE 7: MEAN % OF URINARY METABOLITES OF INDAN*

SAMPLE	1-INDANONE	2-INDANONE	2-INDANOL	5-INDANOL	2-OH-1-ONE	CIS-DIOL	3-OH-1-ONE	TRANS-DIOL
M 24	2.2 +/- 1.4	4.9 +/- 2.2	4.2 +/- .7	1.2 +/- .2	31.5 +/- 10	6.3 +/- 3.4	41.8 +/- 8	7.8 +/- 1.7
F 24	2.7 +/- .6	7.1 +/- 3.1	1.6 +/- .4	1.0 +/- .3	35.5 +/- 6	17 +/- 6	31.5 +/- 4	5.7 +/- 2
M 48	2.7 +/- .4	3.2 +/- 1.1	1.1 +/- .2	1.1 +/- .3	50 +/- 8	4.3 +/- 2	27.8 +/- 5	9.1 +/- 2
F 48	3.0 +/- 1.6	2.3 +/- .4	4.1 +/- 1.2	1.6 +/- .6	41.1 +/- 9	7.2 +/- 4.3	29.7 +/- 4	10 +/- 3
M 14D	1.7 +/- .6	5.2 +/- 1.2	2.7 +/- .3	1.1 +/- .2	30.2 +/- 3	3.3 +/- 2	51.2 +/- 2	4.5 +/- 1
F 14D	3.8 +/- 2.2	3.4 +/- 1.2	4.8 +/- .9	1.0 +/- .2	19.4 +/- 3	5.9 +/- 2.2	54.0 +/- 5	4.8 +/- 1

*EXCLUDING 1-INDANOL

Comparing the sites of metabolic oxidation in indan to that found for tetralin, one can see similarities and differences. For both indan and tetralin the preferred ring for metabolic oxidation appeared to be the non-aromatic ring, the only exception being the modest amount of 5-indanol present in the urine of rats dosed with indan. The simple ketones, 1-indanone and 2-indanone, were isolated as metabolites from indan but the analogous compounds 1-tetralone and 2-tetralone were not found as metabolites from tetralin. Also, 1,4-tetralindiol was observed to be present in tetralin exposed rats while in indan exposed rats, no 1,3-indandiol was detected. Both compounds yielded two hydroxy-ketones, a 2-hydroxy-1-ketone and a metabolite in which one benzylic position had been oxidized to a ketone while the other benzylic carbon had been converted to an alcohol.

The metabolic pathway to the two hydroxy-ketone derivatives of indan can be illustrated in several ways. (Figure 12A) Indan (I) can be hydroxylated in the C-1 position to give 1-indanol (II). This can then be hydroxylated again to create either isomer of the diol (IV) or oxidized to produce the ketone (III). The ketone, III can then be hydroxylated at C-2 to yield the 2-hydroxy-1-indanone (V). The diol, IV, can be oxidized at the C-1 position to also give V. The reverse of this reaction, the reduction of V to create IV is also known (Lewis, 1966).

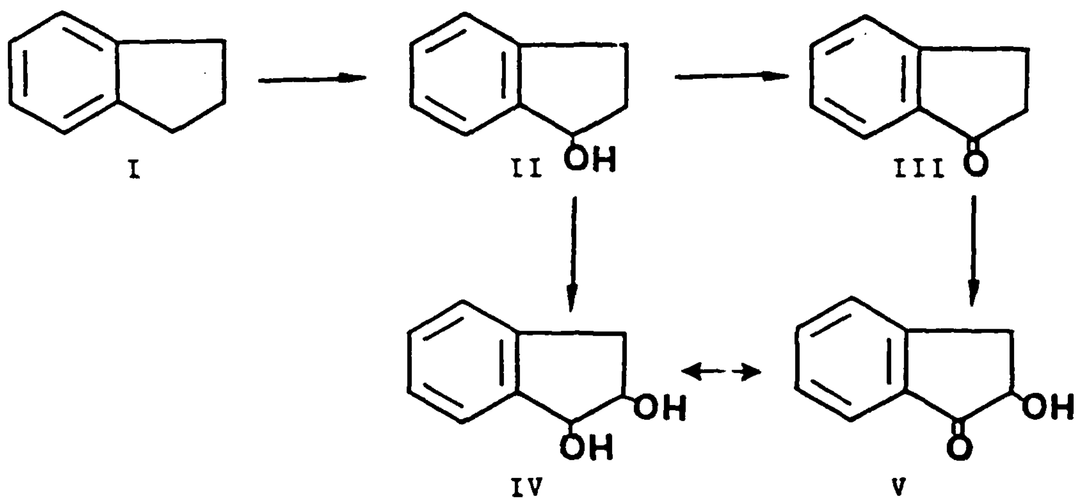


Figure 12A Possible Pathway For 2-Hydroxy-1-Indanone

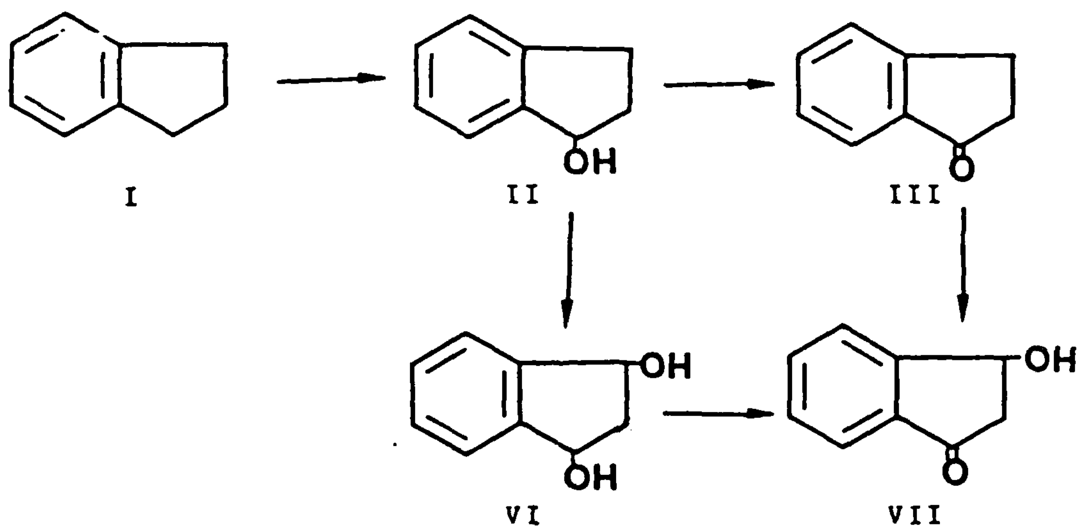


Figure 12B Possible Pathway for 3-Hydroxy-1-Indanone

A similar pathway can be constructed for the formation of 3-hydroxy-1-indanone, (Figure 12B). I can be hydroxylated at the C-1 position to produce II. Oxidation of II could create III, which is followed by hydroxylation at C-3 to form the hydroxy-ketone (VIII). Alternatively, II can be hydroxylated at C-3 to give VI, which is then oxidized to produce VII. The latter reaction must occur rapidly since no 1,3-indandiol was detected as a metabolite of indan.

SYNTHESIS

Two metabolites in the urine were characterized as hydroxy-ketones by GC/MS. Therefore, all the hydroxy-1-indanone derivatives were synthesized using various techniques. The mass spectra for all these compounds are shown in figures 13-18 and their principle fragmentations are shown in table 8.

The first approach to the synthesis of 5-hydroxy-1-indanone was according to Ingold (1923) starting with m-methoxy-3-phenylpropionic acid. The Friedel-Crafts ring closure followed by treatment with H_3O^+ was used to give the major product, 5-hydroxy-1-indanone, and the minor products, 7-hydroxy-1-indanone and 5-methoxy-1-indanone. GC/MS showed only the presence of the minor products. Several unsuccessful attempts were made to cleave the ether function of the methoxy derivative. Finally, using the starting material, 5-methoxy-1-indanone and aluminum bromide, a compound was produced with a melting point nearly corresponding to the literature value for the desired product. Analysis by GC/MS failed to give any product from this method. It was theorized 5-hydroxy-1-indanone did not have enough volatility to elute from the GC column. A direct-insertion probe into the MS verified the presence of 5-hydroxy-1-indanone.

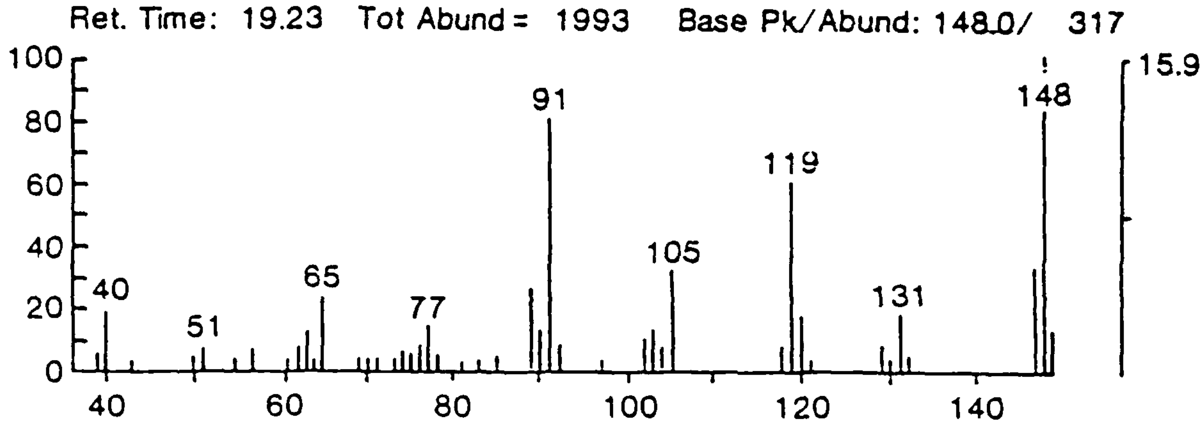


Figure 13 : 2-Hydroxy-1-indanone

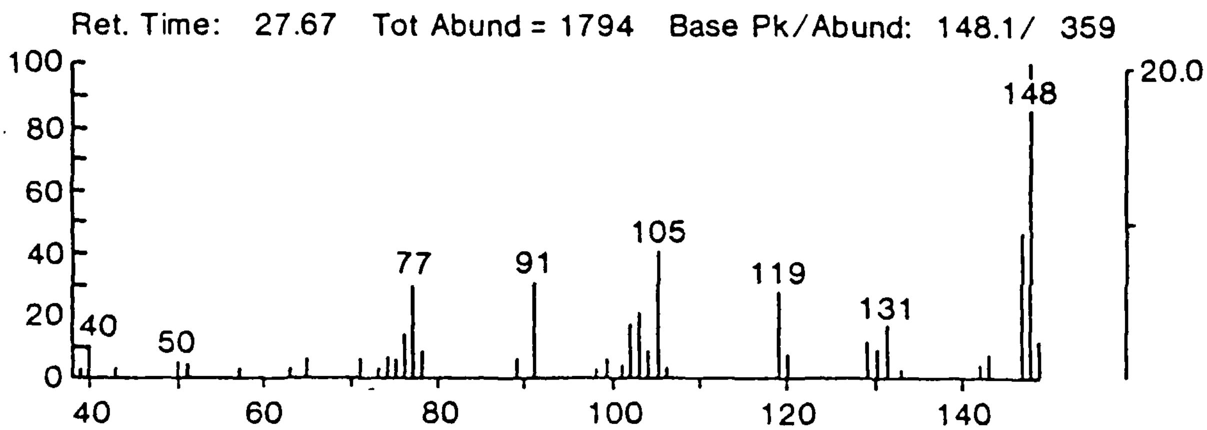


Figure 14 : 3-Hydroxy-1-indanone

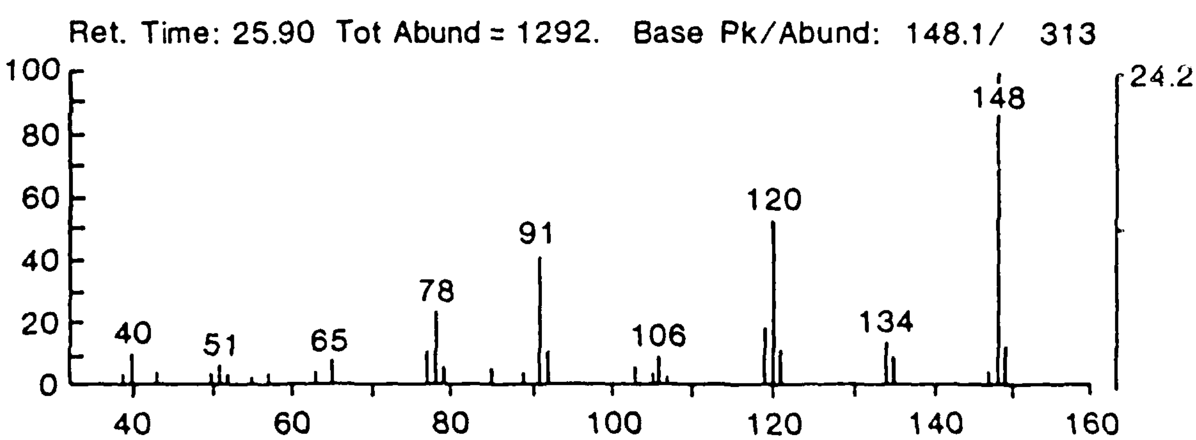


Figure 15 : 4-Hydroxy-1-indanone

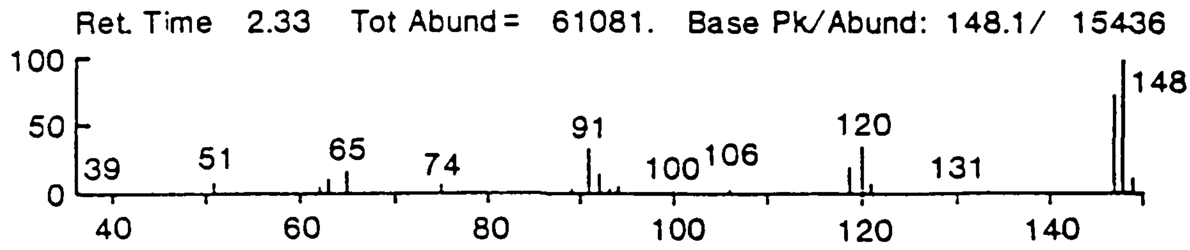


Figure 16 : 5-Hydroxy-1-indanone

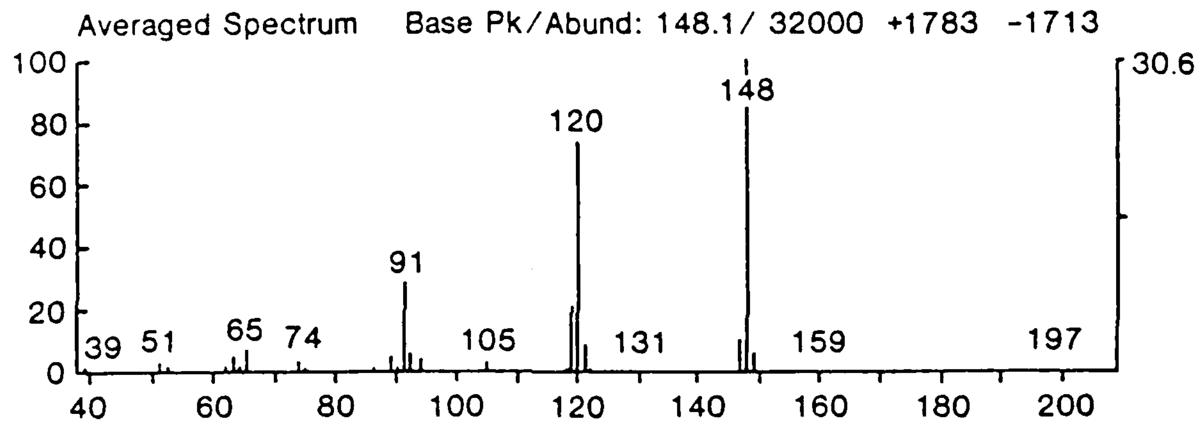


Figure 17 : 6-Hydroxy-1-indanone

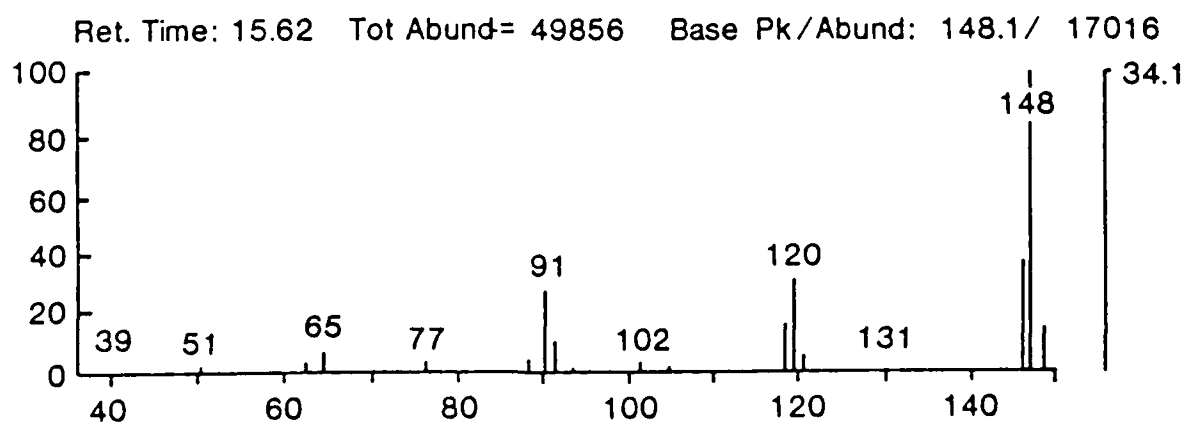


Figure 18 : 7-Hydroxy-1-indanone

It was observed in the synthesis of 3-hydroxy-1-indanone that the intermediate, 3-bromo-1-indanone, was very unstable. Attempts to purify this intermediate led to the elimination of HBr to give 1-indenone.

TABLE 8: PRINCIPLE FRAGMENTATIONS OF THE HYDROXY-1-INDANONES AND THEIR RELATIVE ABUNDANCES IN MASS SPECTRA

m/z	2-OH	3-OH	4-OH	5-OH	6-OH	7-OH
149	12.9	12.3	12.8	11.3	6.5	10.3
148	100	100	100	100	100	100
147	33.4	47.6	5.1	80.3	10.4	37.7
131	17.7	16.7	-	1.0	1.0	1.9
121	3.8	-	11.2	6.3	8.0	5.3
120	18.3	8.1	53.4	34.2	73.4	30.5
119	60.6	27.3	19.8	19.1	21.0	16.0
105	31.5	39.8	4.5	2.7	2.8	2.2
104	6.9	8.4	-	0.2	-	0.2
91	80.8	30.1	41.5	31.4	27.5	27.6
90	13.2	-	-	1.6	1.7	1.3
89	26.2	5.3	4.2	5.1	4.4	3.5
78	5.0	8.4	23.6	1.0	1.4	0.7
77	14.2	29.5	10.9	2.9	1.6	2.2
76	8.8	13.9	-	0.8	1.6	0.8

Synthesizing 2-hydroxy-1-indanone was approached by two different methods. The first utilized the silyl enol ether derivative which produced the desired compound in approximately 5% yield. The next attempt was replacement of the bromine in 2-bromo-1-indanone. This also created the product but the yield was also low.

Several attempts were made to synthesize the 1-hydroxy-2-indanone via the silyl enol ether and the 1-bromo-2-indanone. The first effort produced the silyl enol ether intermediate in approximately 65% yield. Attempts to hydroxylate the silyl enol ether primarily regenerated the starting material, 2-indanone.

The next effort to prepare 1-hydroxy-2-indanone consisted of the synthesis of 1-bromo-2-indanone using N-bromosuccinimide. This intermediate was unstable, eliminating HBr readily. However, a sufficient amount was produced to try to hydrolyze to the 1-hydroxy-2-indanone.

The nuclear magnetic resonance spectra of the purchased or synthesized compounds identified as metabolites is shown in the following pages (figures 19-25) with the exception of the hydroxy indanones.

Looking at the spectra of 1-indanol and 2-indanol (figure 19 and 20), one can easily distinquish the sharp singlet from the hydroxylic proton. For 2-indanol, the signal is at 2.4 ppm while the hydroxylic absorption of 1-indanol is shifted slightly downfield to 2.6 ppm. The methine proton chemical shift occurs about 5.05 ppm and 4.6 ppm for 1-indanol and 2-indanol, respectively.

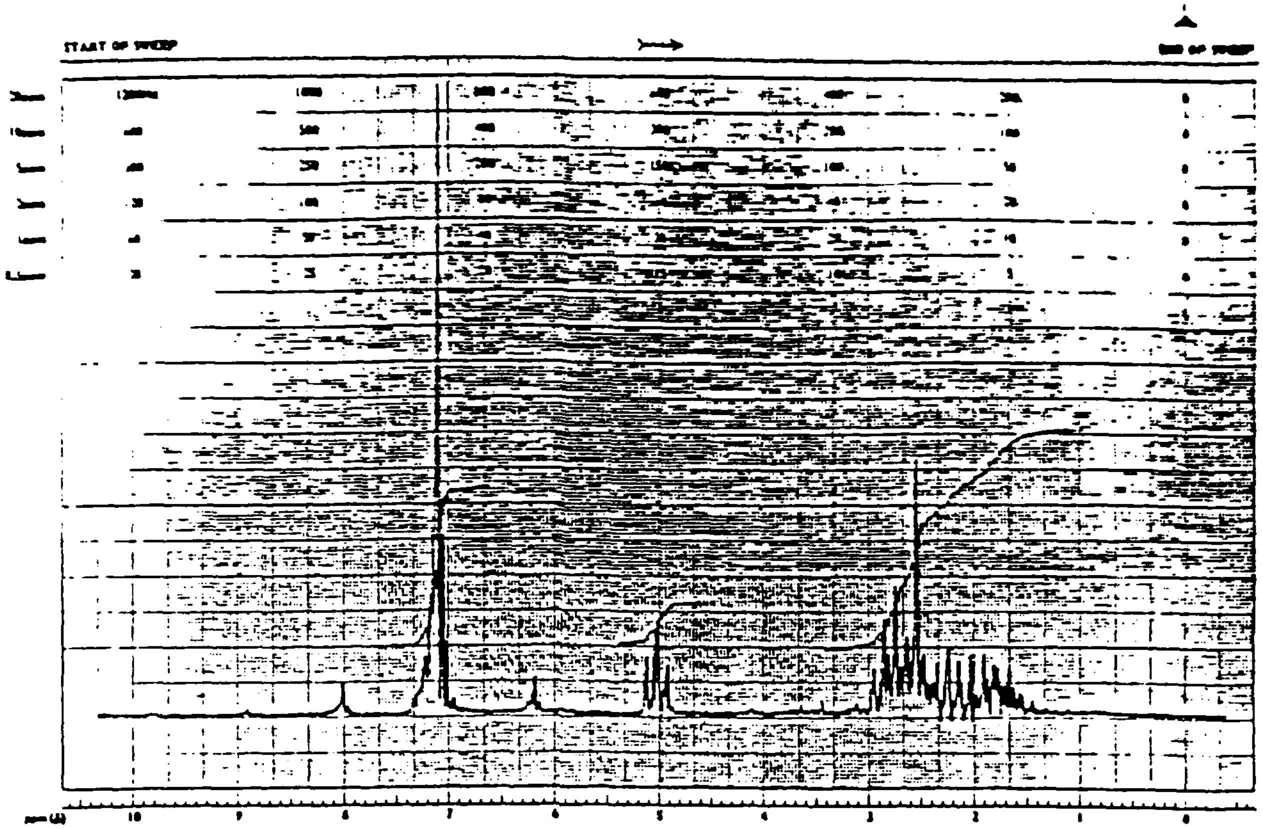


Figure 19: NMR Spectra for 1-Indanol

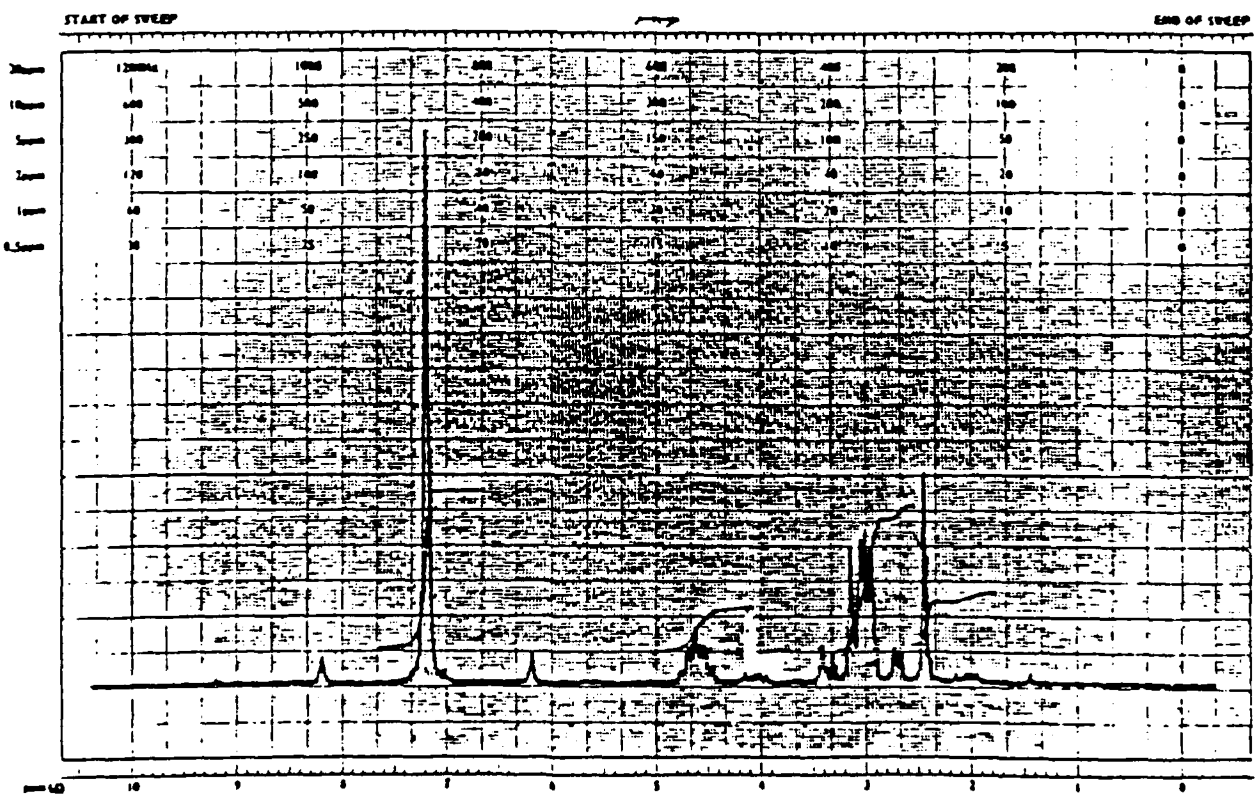


Figure 20: NMR Spectra for 2-Indanol

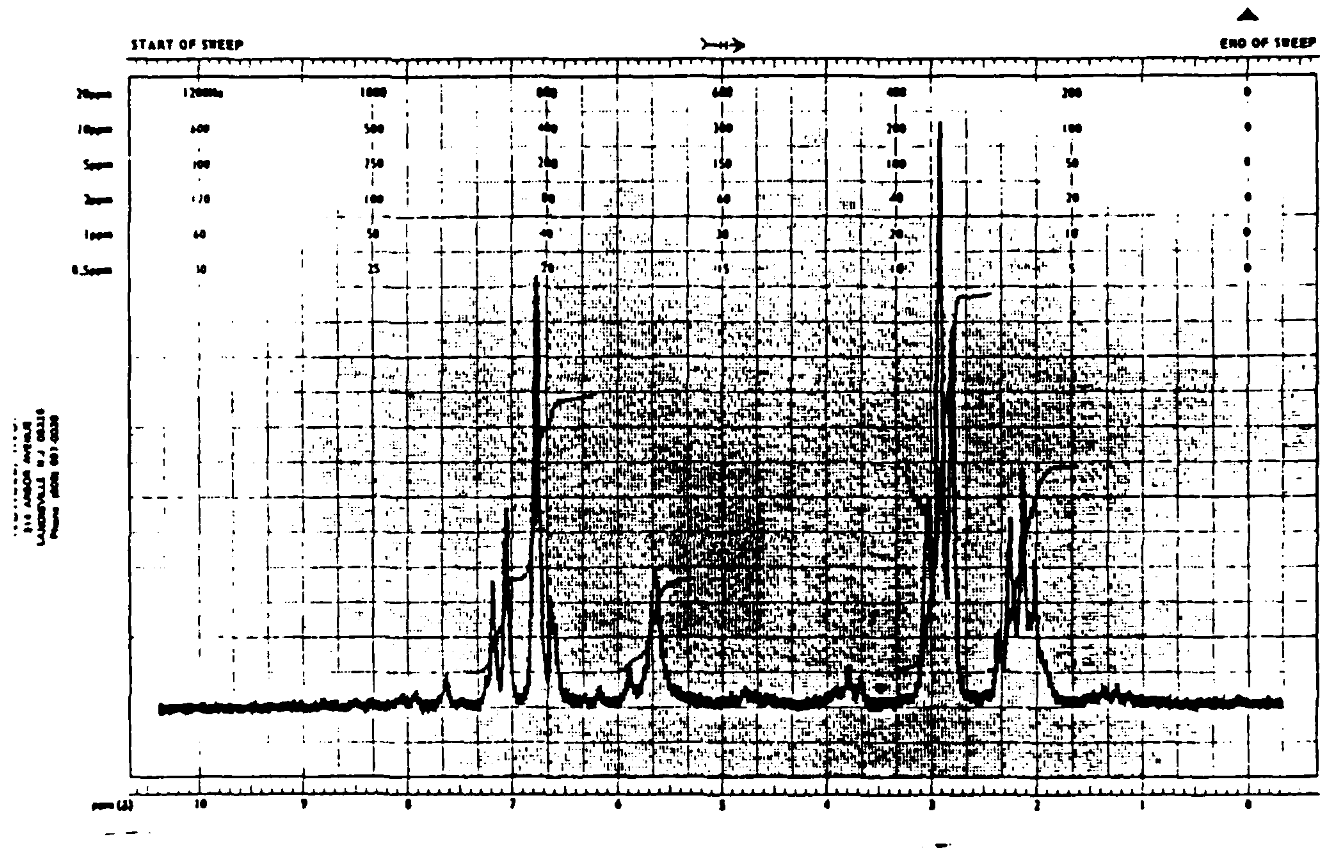


Figure 2.1 : NMR Spectra for 5-Indanol

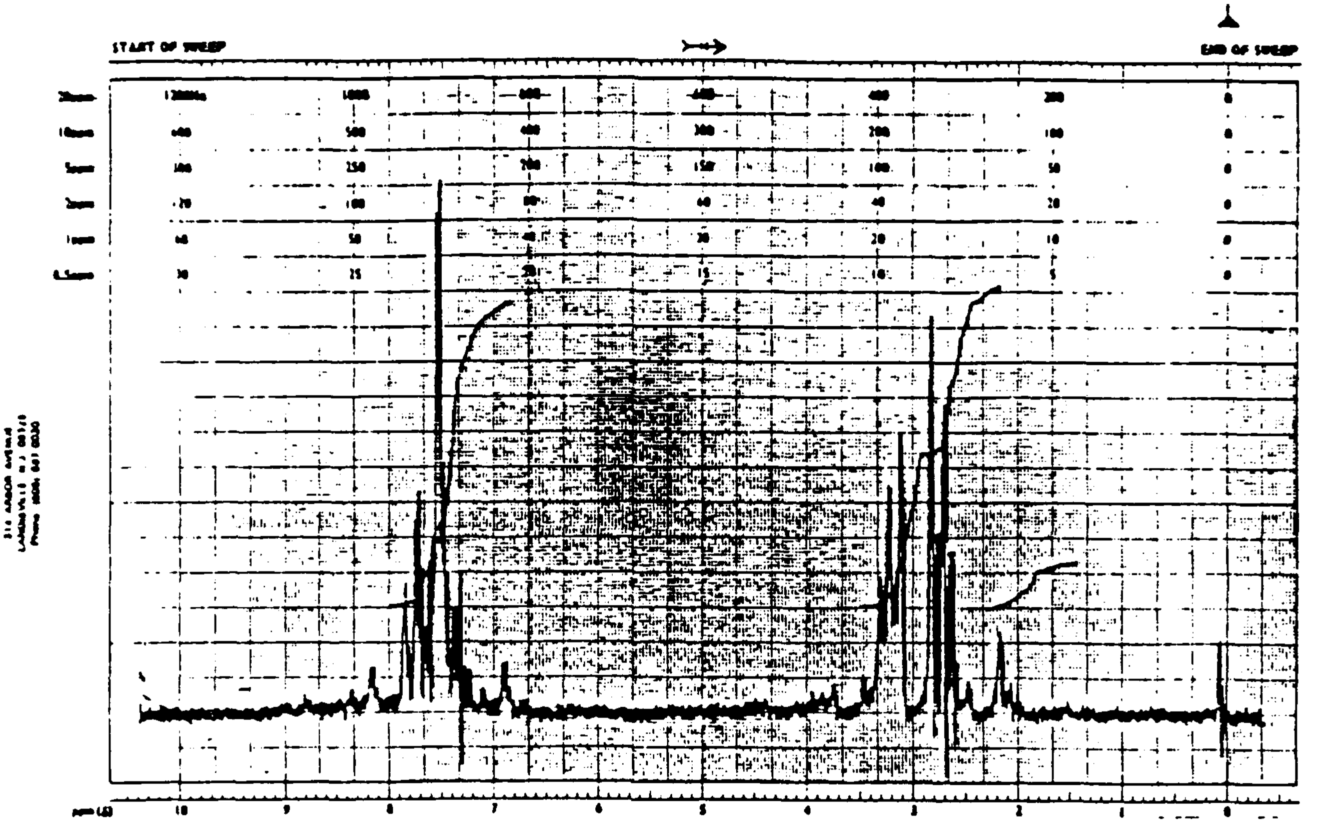


Figure 22. NMR Spectra for 1-Indanone

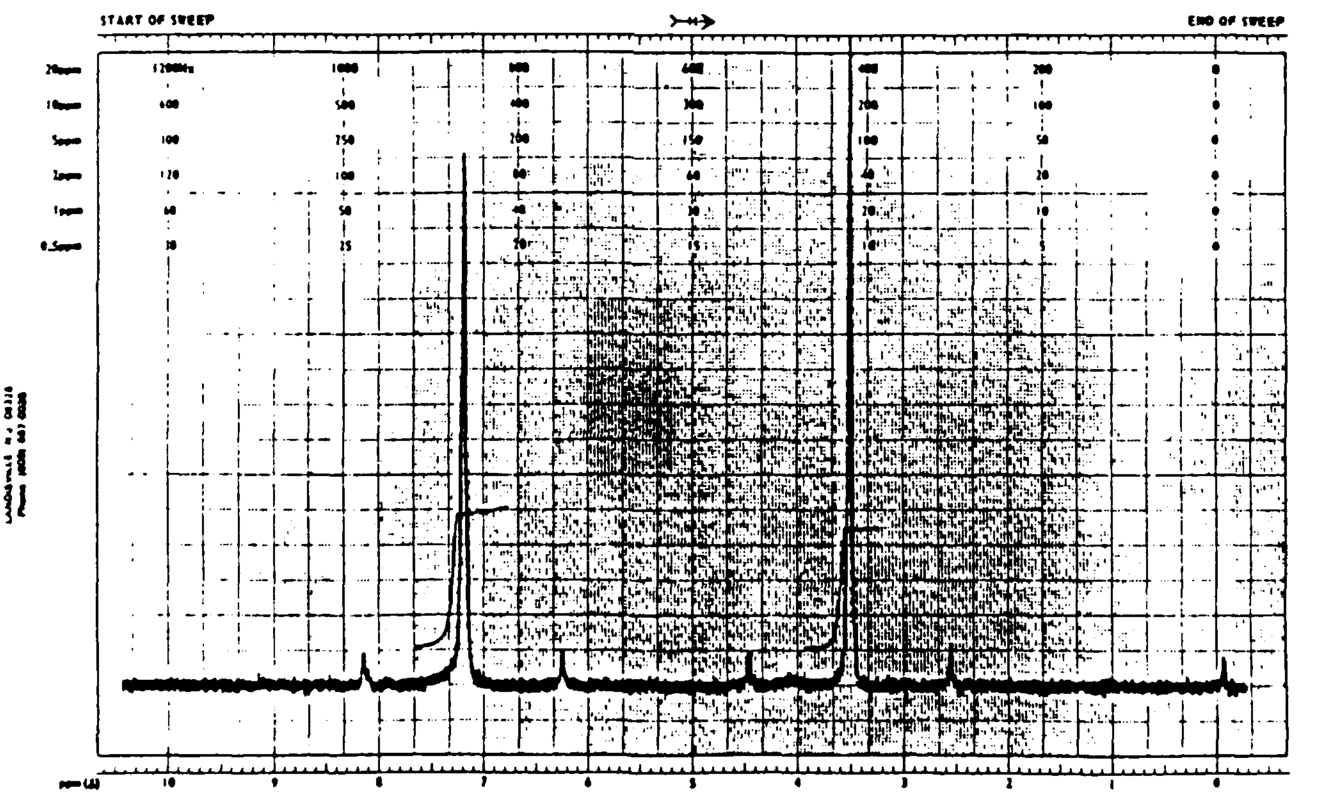


Figure 23 : NMR Spectra for 2-Indanone

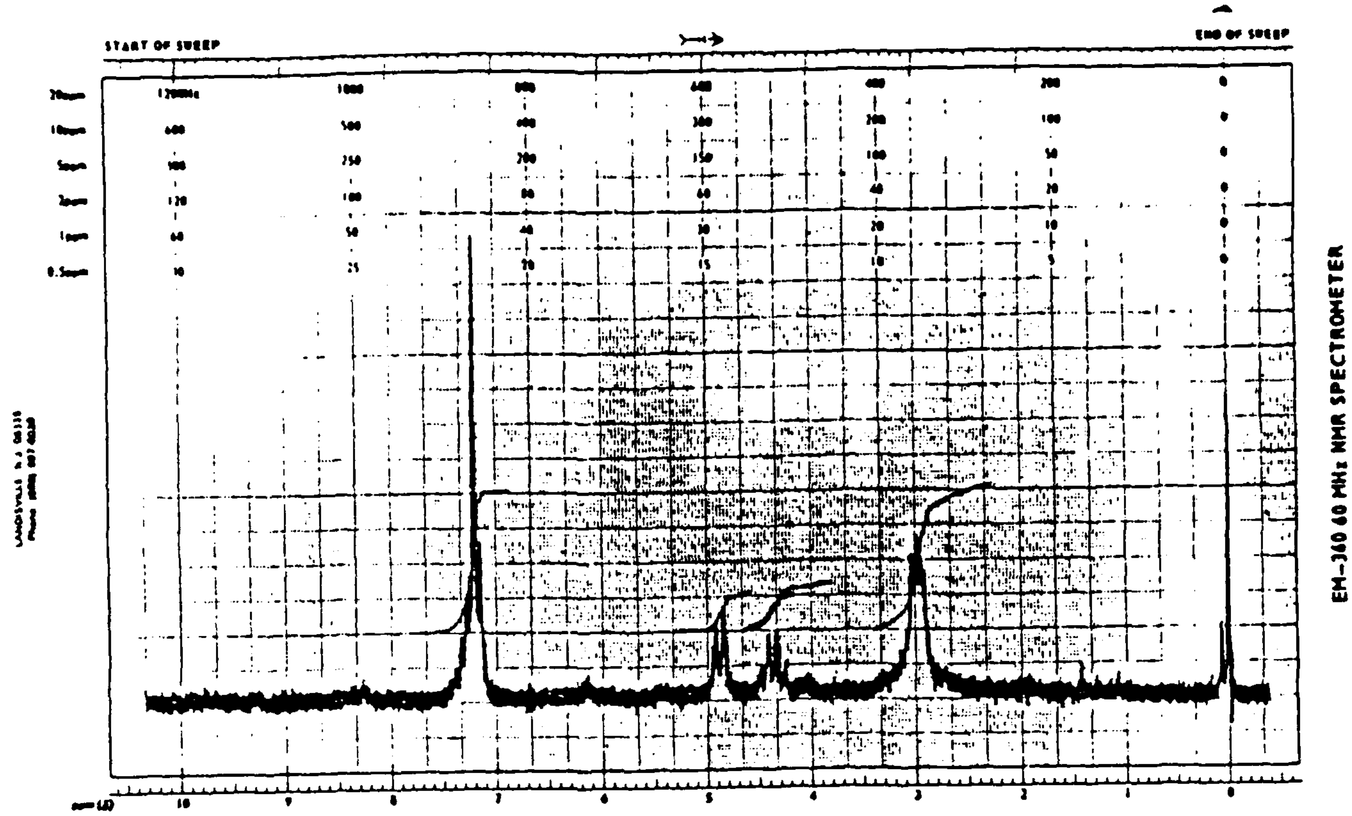


Figure 24. NMR Spectra for cis-1,2-Indandiol

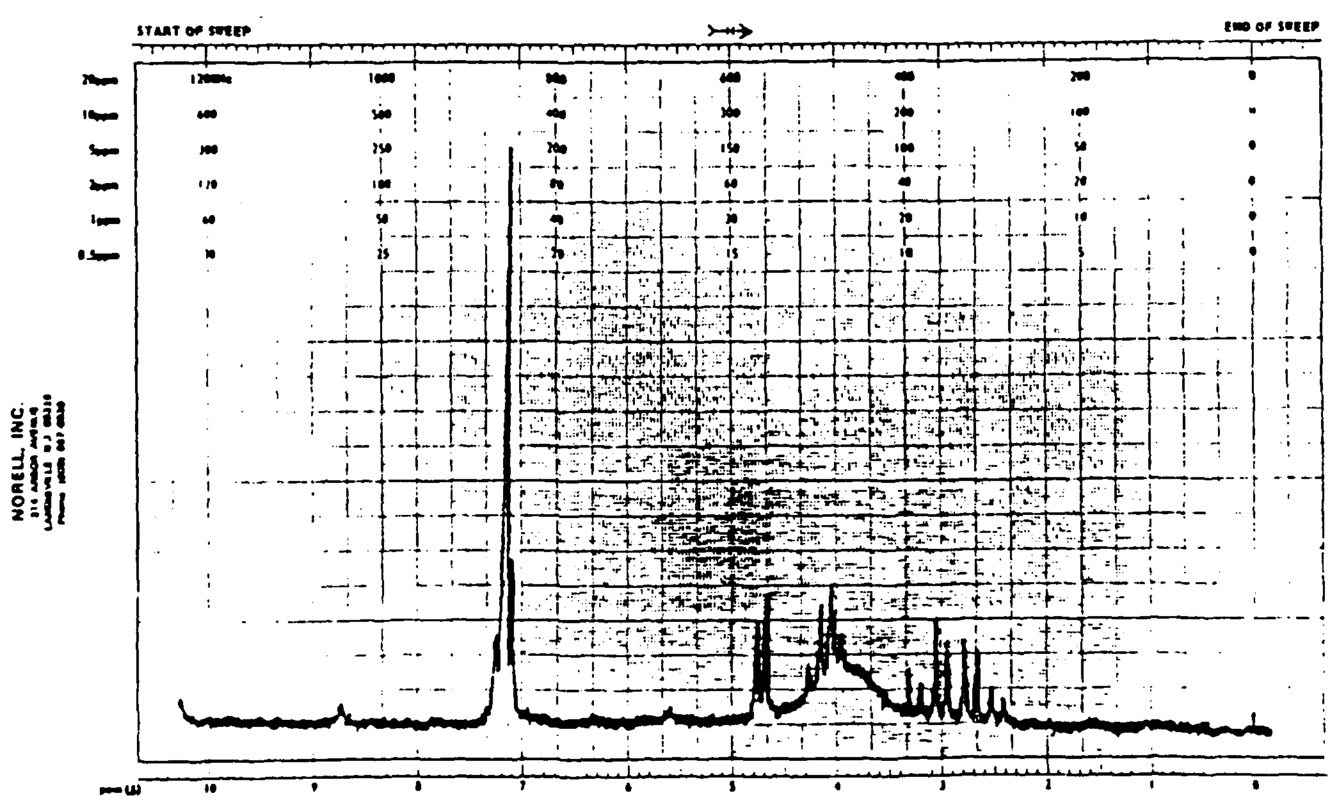
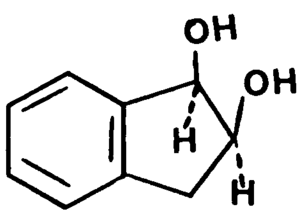


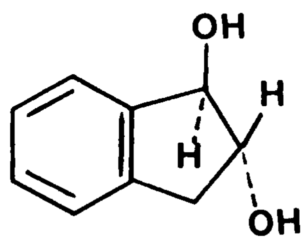
Figure 25 NMR Spectra for trans-1,2-Indandiol

The hydroxylic proton for the phenol, 5-indanol, apparently is not hydrogen bonded since the broad absorption occurs at 5.65 ppm (figure 21).

The spectra for the cis and trans isomers of 1,2-indandiol are interesting. Because of the planarity of the molecule, the methylene protons on C-3 are not equivalent (see figure 26). For the cis isomer, Ha and Hb are multiplets centered around 3.0 ppm. Hc and Hd appear at 4.9 ppm and 4.45 ppm, respectively. To explain the multiplet for Ha, Hb, and Hc of the trans isomer, an eight line spectra (doublet of doublets of doublets) would occur if J_{AC} did not equal J_{BC}. However if J_{AC} equals J_{BC}, then the splitting would simplify to a doublet of doublets (four line spectra). This seems to be shown by multiplet centered around 2.9 ppm.

Figure 26. Cis-1,2-indandiol Trans-1,2-indandiol





METABOLITES OF RATS GIVEN ENZYME MODIFIERS

Xenobiotics are metabolized by enzyme systems present predominantly in the liver; however, kidney, lung, brain, intestine, and skin also contain these enzyme systems (Estabrook, 1982). These systems are collectively known as the mixed function oxidase system

(MFO) and serve to increase the water solubility of the xenobiotic and speed its elimination from the body. The terminal oxidase of the MFO system is a group of hemeproteins called P-450. During metabolism, many xenobiotics can inhibit or induce the capacity of the MFO system to metabolize foreign compounds. Metabolic conversion of a xenobiotic can result in the formation of products that are either less toxic or more toxic than the parent compound. Therefore, when metabolism decreases toxicity, enzyme induction can be protective and enzyme inhibition detrimental or conversely, when metabolism increases toxicity, enzyme inhibition can be protective and enzyme induction detrimental.

To determine what effects inhibition or induction of the MFO system might have on tetralin metabolism and toxicity, enzyme modifiers were given to rats prior to tetralin exposure. The three enzyme modifiers administered were phenobarbital, 3-methylcholanthrene, and SKF 525-A. Rats pretreated with corn oil served as controls.

Phenobarbital, which is actively metabolized by the liver MFO, increases the amount of cytochrome P-450 and other associated MFO enzymes in the liver cell (Hodgson, 1982). However, phenobarbital does not induce renal MFO activity in the rat or mouse. Pretreatment of rats with 3-methylcholanthrene causes an increase in hepatic and renal cytochrome P-448 levels (Casarett and Doull, 1980). Finally, SKF 525-A interferes with the binding of substrates to cytochrome P-450 and reduces the rate of drug metabolism (Goodman and Gilman, 1980). The absence or presence of tetralin metabolites in urine and kidney extracts for all exposure groups and the corresponding renal pathology is summarized in Table 9.

Relative amounts of tetralin metabolites for positive control male rats pretreated with corn oil before tetralin dosing and male rats pretreated with sodium phenobarbital and SKF 525-A compared closely with male rats exposed only to tetralin. However, the relative amounts of metabolites detected from male rats pretreated with 3-methylcholanthrene were different than those from other pretreated male rats and controls. All urine samples from 3-methylcholanthrene pretreated male rats had lower amounts of 2-hydroxy-1-tetralone and slightly increased levels of 4-hydroxy-1-tetralone in 48 hour and final two-week urine samples when compared to positive controls and other pretreated male rats. There was also an increase in the levels of 1,2-tetralindiol in all urine samples from 3-methylcholanthrene pretreated male rats.

When compared to female tetralin-exposed rats, female positive control rats had more 4-hydroxy-1-tetralone in their urine samples. Female rats pretreated with SKF 525-A had similar relative amounts of metabolites as did the female positive control rats except for a slight increase in 2-hydroxy-1-tetralone. Compared to female positive control rats, female rats pretreated with sodium phenobarbital showed a decrease in the relative amounts of 2-tetralol and 4-hydroxy-1-tetralone in 24 hr and 48 hr urine samples. 3-Methylcholanthrene pretreated female rats had decreased levels of 2-tetralol in all urine samples and decreased 2-hydroxy-1-tetralone in their 48 hr urine samples but in contrast had increased levels of 4-hydroxy-1-tetralone in their 48 hr and final urine samples.

TABLE 9. TETRALIN METABOLITES DETECTED IN URINE AND KIDNEY EXTRACTS AND CORRESPONDING RENAL PATHOLOGY

GROUP & TREATMENT	EXPOSURE ROUTE AND DOSE	URINARY METABOLITES		KIDNEY METABOLITES		RENAL LESIONS	
		MALE	FEMALE	MALE	FEMALE	MALE	FEMALE
1. Negative Control Water	0.5 mL/kg i.g.	NO	NO	NO	NO	YES (Min/Mild)	NO
2. Tetralin	0.5 mL/kg i.g.	YES[a]	YES[a]	NO	NO	YES (Moderate)	NO
Enzyme Modifiers							
3. Positive Control Corn Oil Tetralin	2.0 mL/kg i.p. 0.5 mL/kg i.g.	YES[a]	YES[a]	YES[b]	NO	YES (Mild)	NO
4. Sodium Phenobarbital Tetralin	102 mg/kg i.p. 0.5 mL/kg i.p.	YES[a]	YES[a]	YES[c]	NO	YES (Mild)	NO
5. SKF 525-A Tetralin	25 mg/kg i.p. 0.5 mL/kg i.g.	YES[a]	YES[a]	YES[d]	NO	YES (Mild)	NO
6. 3-MC Tetralin	27 mg/kg i.p. 0.5 mL/kg i.g.	YES[a]	YES[a]	YES[e]	NO	YES (Mild)[e]	NO

a = 1-tetralol, 2-tetralol, 2-hydroxy-1-tetralone, 4-hydroxy-1-tetralone,1,2-tetralindiol and 1,4-tetralindiol
b = tetralin, 1-tetralol, 2-tetralol and 1-tetralone
c = 1 rat had 1-tetralol and 1-tetralone, 2 rats had tetralin, 1-tetralol, 2-tetralol and 1-tetralone
d = 1 rat had 1-tetralol and 1-tetralone, 2 rats had tetralin, 1-tetralol, 2-tetralol and 1-tetralone
e = 1 rat had 1-tetralol and 1-tetralone and exhibited renal lesions the other two rats had no metabolites and no renal lesions

Tetralin metabolites were not detected during GC/MS analysis of the kidney extracts taken from female positive control rats or female rats pretreated with sodium phenobarbital, 3-methylcholanthrene or SKF 525-A. However, tetralin, 1-tetralol, 2-tetralol, and 1-tetralone were detected in the extracts from positive control male rats and male rats pretreated with sodium phenobarbital and SKF 525-A. The appearance of renal lesions and a ketone metabolite in kidney extracts of pretreated male rats is noteworthy when non-pretreated male rats that also exhibited renal lesions had no ketone metabolite present in their kidney extracts. One male rat pretreated with sodium phenobarbital and one male rat pretreated with SKF 525-A showed no tetralin or 2-tetralol in their kidney extracts, but did have 1-tetralol and 1-tetralone. One male rat pretreated with 3-methylcholanthrene had only 1-tetralol and 1-tetralone detected in its kidney extract. This male rat was also the only 3-methylcholanthrene pretreated rat to exhibit renal lesions. Tetralin metabolites were not detected in the two 3-methylcholanthrene pretreated rats which did not demonstrate renal lesions. Because these tetralin metabolites were present in kidney extracts in only small amounts, consistent gas chromatographic detection of them was difficult. Therefore, for the animals in which metabolites were detected, only relative amount comparisons of the major metabolites, 1-tetralol and 1-tetralone, were performed. For positive control male rats and male rats pretreated with sodium phenobarbital or SKF 525-A, the relative amount percentages of 1-tetralol and 1-tetralone were 49.8 $\pm$ 7.78 and 50.2 $\pm$ 7.78, respectively. Although tetralin metabolites were present in the kidney extract of one male rat pretreated with 3-methylcholanthrene, it is interesting to note the relative amounts of 1-tetralol and 1-tetralone were 82% and 18%, respectively. Differences between the type of metabolite detected in kidney extracts of pretreated and non-pretreated male rats, and the absence of renal lesions and metabolites in two 3-methylcholanthrene pretreated male rats, suggest induction or that inhibition of the MFO enzyme system affects the metabolism of tetralin and possibly alters its toxicity. Therefore, the investigation of cyclic hydrocarbon metabolism following enzyme modification should be continued.

IV. CONCLUSION

The purpose of this research was to further investigate the toxic effects of cyclic hydrocarbons in rats. By using the hydrocarbon, indan, to compliment earlier research on jet fuel, JP-10, cis- and trans- decalin, and tetralin, it was anticipated some enlightenment could be obtained on the nephrotoxicity mechanism. It appears the structural differences between decalin, tetralin, and indan do not affect the nephrotoxicity of cyclic hydrocarbons. In addition, the isolation of hydroxy ketones as urinary metabolites for all the cyclic hydrocarbons may indicate that a difunctional molecule is the reactive nephrotoxic metabolite. It has been suggested that the presence of ketones in the kidney extracts could represent a chemical marker of renal damage (Olson et. al., 1986). This study of indan would support this theory.

Several conclusions can be drawn from the investigation of indan. Exposure to the hydrocarbon causes weight loss in both sexes. Also, indan seems to be mildly nephrotoxic to male rats. It is not known whether it is the hydrocarbon itself or a metabolic product that reacts with the protein alpha 2u globulin or interferes with the degradation of the protein to cause the renal lesions. Further work is needed to clarify the specific mechanisms which these hydrocarbons operate through to produce the kidney damage.

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